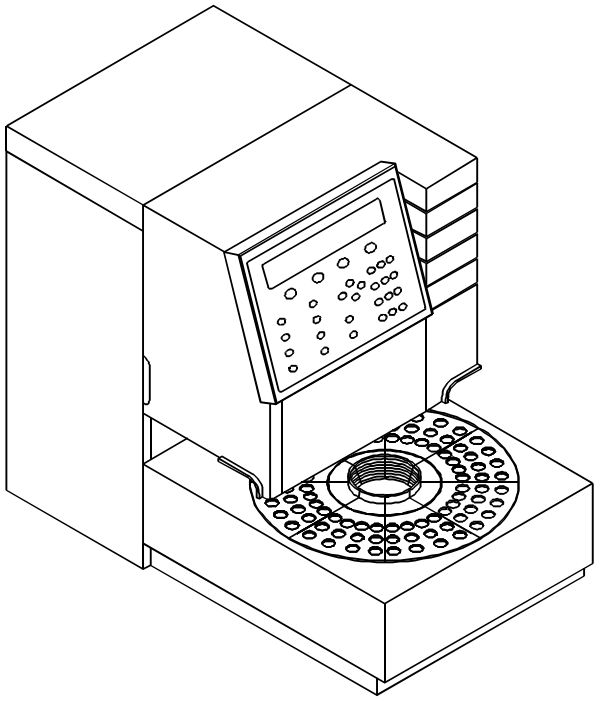
# Triathlon

## *user manual*



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HOLLAND
Spark

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CE

# DECLARATION OF CONFORMITY

We *Spark Holland BV,*
Pieter de Keyserstraat 8,
NL-7825 VE Emmen.

Declare that the product:

---

**Autosampler "Triathlon", type 900**

---

is in conformity with the following documents:

- EEC directives 89/392 incl. 91/368 and 93/44 (machine safety) and EEC directives 73/23 and 93/68 (low voltage safety), applied with the following standard:

  EN 61010-1 Safety requirements for laboratory equipment (Class I, Installation cat. II, Pollution degree II)

WARNING

***Spark Holland will not accept any liability for damages directly or indirectly caused by connecting this instrument to devices which do not meet relevant safety standards.***

- EEC directives 89/336 and 92/31 (EMC requirements, applied with the following standards:

  EN 50081-1 Generic emission standard
  EN 50082-1 Generic immunity standard
  EN 61000-3-2 Harmonic current emissions

***Use shielded cables and connectors for all remote connections.***

Emmen, October 1998. Rein van den Berg, QA manager.

# *About this manual*

This manual has been written for laboratory technicians who use the Triathlon for execution of analytical runs. It is assumed that the user of this manual has basic knowlegde of how to use menu-driven software and that she/he is familiar with standard laboratory and HPLC terminology.

Chapters 1 and 2 of this manual contain basic information that should be read by every user of the Triathlon:

- Chapter 1 identifies main parts of the Triathlon.
- Chapter 2 explains how to install parts either for maintenance purposes or for preparing the Triathlon for an analytical run.

For first-time users:

- Chapter 3 explains the main menus of the Triathlon and offers a basic explanation of how to operate the Triathlon.
- Chapter 4 offers a number of examples that teach the user how to work with the Triathlon.

For experienced users:

- Users who understand how the Triathlon works will probably only use the reference part of this manual (Chapter 5) to look up the purpose of a particular function.
- Experienced users may find the overview of programming options (Appendix G) useful for quick reference of possibilities of the Triathlon.

The appendices in this manual offer specialist information.
An index has been provided to allow the user to find required information quickly.

## *Typographical conventions*

The following typographical conventions are used in this manual:

| | |
|---|---|
| Initial Capitals | are used for names of the menus: Ready Menu, Series Menu etc. |
| <CAPITALS> | are used to refer to soft function keys |
| **bold type** | is used for names of keys in the keypad |
| [0100] | is used to indicate values that have to be entered with the numerical keypad |

## *Symbols*

The following symbols are used on the Triathlon:

This sticker indicates that care should be taken to prevent personal injury or damage to parts of the Triathlon.

This sticker (with yellow background color) at the back of the Triathlon calls attention to the fact that you are expected to consult this manual for instructions on how to operate the Triathlon.

The following pictograms are used in this manual:

calls attention to a procedure, which, if not correctly executed, could result in injury or loss of life. Do not proceed beyond a "DANGER" sign until the indicated conditions are fully understood and met.

calls attention to a procedure, which, if not correctly executed, could result in personal injury. Do not proceed beyond a "WARNING" sign until the indicated conditions are fully understood and met.

calls attention to a procedure, which, if not correctly executed, could result in damage to the equipment. Do not proceed beyond a "CAUTION" sign until the indicated conditions are fully understood and met.

calls attention to important information. Read this information before continuing.

## *Safety practices*

The following safety practices are intended to ensure the safe operation of the equipment.

- Removal of panels may expose users to dangerous voltages. Disconnect the Triathlon from all power sources before removing protective panels.
- Always replace blown fuses with fuses of the size and rating indicated on the fuse panel and holder. Refer to Appendix B of this manual for more information on fuses.
- Replace or repair faulty insulation on power cords.
- Check that the actual power voltage is the same as the voltage for which the Triathlon is wired. Make sure power cords are connected to correct voltage sources.
- The Triathlon must only be used with appliances and power sources with proper protective grounding.

- Perform periodic leak checks on supply lines.
- Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of flammable and/or toxic solvents through the municipal sewage system
- Using the Triathlon in other ways than indicated in the instructions given in this manual may cause unsafe conditions.

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# Chapter 1. Introduction

The Triathlon is an autosampler that offers a wide variety of possibilities for HPLC. It incorporates among other things the following features:

- suitable for use with various types of vials by various manufacturers
- capable of executing flushed loop injections, partial loopfill injections and µL pickup (see section 1.1 below)
- accomodates use of various types of syringes (volumes of 100, 250, 500, 1000 µL, or 10 mL)
- fully compatible with other laboratory equipment
- apparatus designed for routine analysis and method development

In addition options like tray cooling and switching valves are available for the Triathlon. These options enhance the uses for the Triathlon considerably (refer to Appendixes B and D for more information on options).

## 1.1 Injection principle

The Triathlon offers three different methods of injection for an analytical run:

| | |
|---|---|
| Flushed loop | The sample loop is completely (quantitatively) filled with sample resulting in extremely good reproducibility. |
| Partial loopfill | The sample loop is partially filled with sample; this means low sample loss and programmable injection volumes. |
| µL pick-up | After aspiration of sample, the sample is transported into the loop with transport liquid (mobile phase); this means no sample loss. |

The Triathlon uses a syringe to aspirate the sample from a vial into the sample loop. To prevent contamination of the syringe the Triathlon is equipped with a buffer tubing between the syringe and the injection valve. Wash solvent is used to remove the sample from the buffer tubing and sample needle, and to rinse the buffer tubing and sample needle. For more technical information on the injection principle used by the Triathlon refer to Appendix H.

For an overview of fluid connections of the Triathlon refer to the illustration inside the cover of the Triathlon.

## *1.2 Front view of the Triathlon*

The front of the Triathlon contains the following elements:

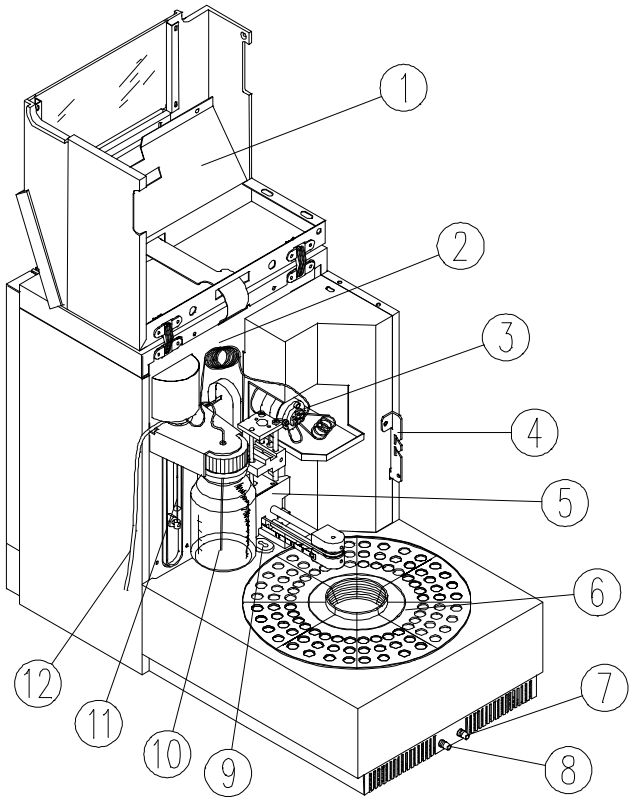



*Figure 1.1 Front view of the Triathlon*

1. Cover (in open position)
2. Buffer tubing
3. Injection valve (Rheodyne)
4. Tubing holder
5. Needle unit
6. Tray with segments
7. Drain wash-position
8. Condensed water and leakage
9. Wash position
10. Wash solvent bottle
11. Syringe
12. Syringe waste tubing

## 1.3 Back view of the Triathlon

The back of the Triathlon contains the following elements:

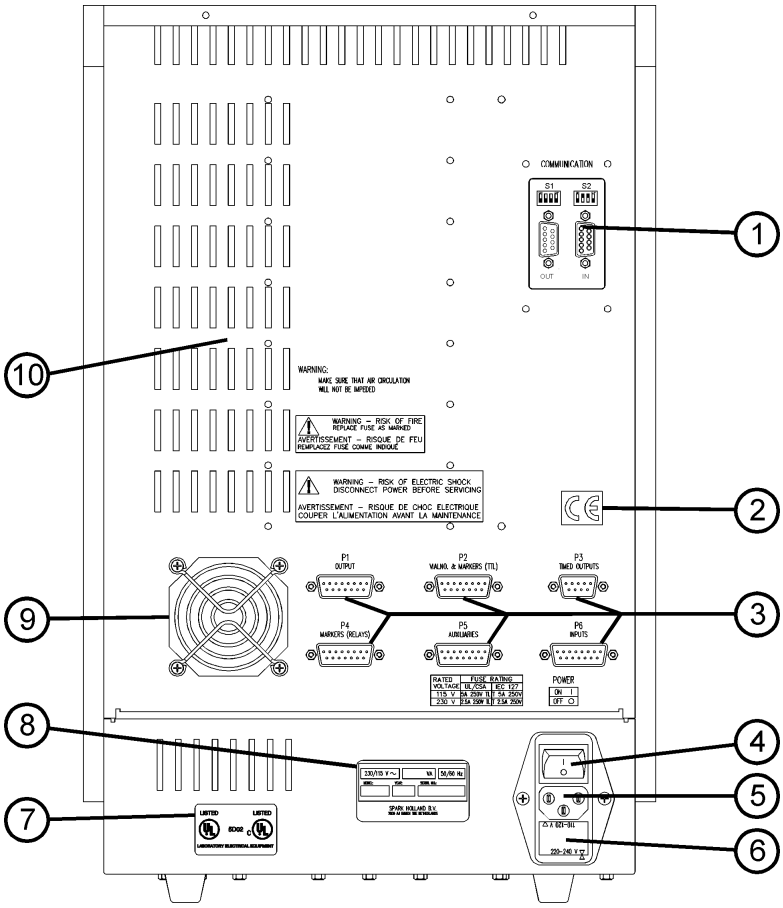



*Figure 1.2 Back of the Triathlon*

1. SparkLink communication interface connector.
2. CE-mark
3. I/O connectors
4. Mains switch
5. Mains input
6. Fuses and voltage selector
7. UL label
8. Type label
9. Fan (if tray cooling is installed)
10. Ventilation holes

## 1.4 Keypad and display of the Triathlon

The keypad of the Triathlon contains the following elements:

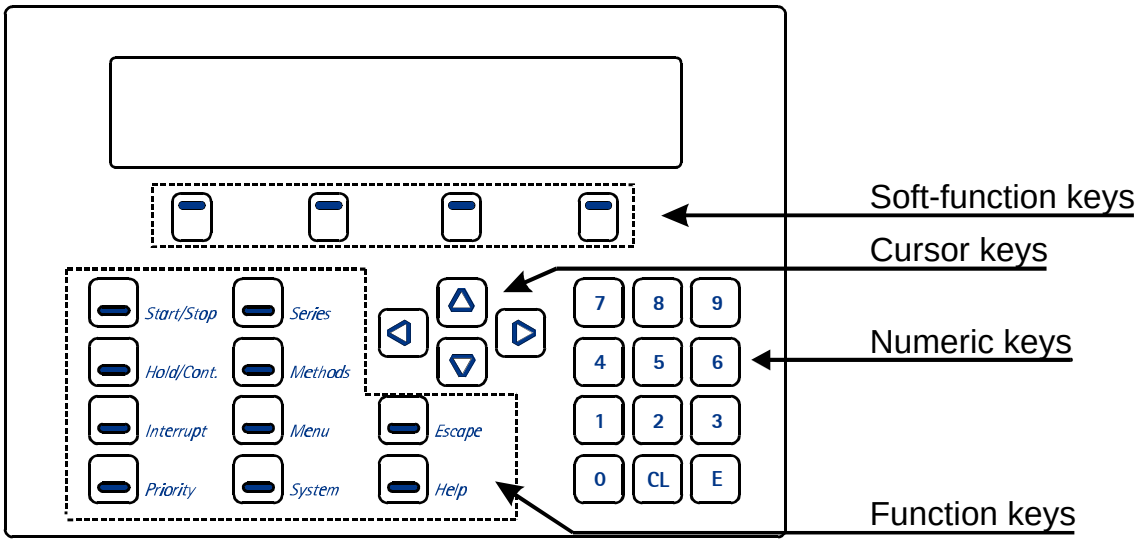



*Figure 1.3 Keypad and display*

**Soft function keys:** the label assigned to these keys depends on the menu that is active. The label of each key is shown in the bottom line of the display.
**Cursor keys**: can be used to move to a different field in the display, to move to a different field in a menu, or to make a displayed value higher or lower.
**Numeric keys**:

- **0** to **9**: to enter numerals in the various programming fields.
- **CL**: to clear a value in a field or replace it by NONE or AUTO.
- **E**: stands for Enter; to go through menu lines or to confirm a choice made in a menu or a value entered. The entered value is checked for validity and then saved.

**Function keys**:

- Run control keys:
  **Start/Stop**: to start or stop automatic processing, or to reset the system after an error has occurred.
  **Hold/Cont**.: to hold or continue the analysis time. The analysis time is extended by the period that Hold is active.
  **Interrupt**: not used.
  **Priority**: to stop a run to process a priority sample before analyzing the rest of the programmed sample series. Before the run is interrupted processing of the present sample will be finished. As soon as the priority sample has been analyzed, the analytical run is resumed. A priority sample is a series of one vial with an injection

method, a wash method and a time base method defined in a template (only possible if the correct settings are entered in the System Menu).

- Programming keys (see also section 3.1 and Chapter 5):
  **Series**: to enter the Series Menu in which series can be defined for an analytical run.
  **Methods**: to enter the Methods Menu in which methods can be programmed for use in an analytical run.
  **Menu**: this key can only be used if [MENU] or [MN] is shown in the top right hand corner of the display. If pressed more possibilities of the menu are displayed.
  **System**: to enter the System Menu in which system settings can be entered.
- General keys:
  **Escape**: allows the user to leave the progamming mode or go to a previous level in the menu. Entered values are checked for validity and then saved.
  **Help**: to display help information; available only for a limited number of functions!

# Chapter 2. Preparations for use

This chapter describes procedures for replacement or installation of parts, either for maintenance or for preparation of the Triathlon for an analytical run.

Please note that the power should **not** be switched off for any of the procedures described below as soft function keys in the Ready Menu must be used.

A number of items required for use of the Triathlon are factory-installed (see Appendix A). However, after the installation procedure described in Appendix A has been executed, check that the following have been correctly installed before you start to use the Triathlon:

- HPLC pump & column connections
- waste tubing
- wash solvent bottle
- syringe
- needle assembly
- syringe, sample loop and buffer tubing
- tray segments and tray types
- reagent vials/transport vials

Loosen the screw at the right-hand side of the cover and lift the cover to be able to execute the procedures described in this chapter.

## 2.1 HPLC connections

Make the following connections:

- HPLC pump to port 1 of the Rheodyne injection valve
- HPLC column to port 6 of the Rheodyne injection valve

The instrument has been flushed with isopropanol before dispatch from the factory. Make sure that the mobile phase of your HPLC system is miscible with isopropanol, or start up with an intermediate solvent as mobile phase (disconnect the HPLC column).

It is very important that the contents of the sample loop are injected in back flush onto the column, therefore: do not exchange column and pump connections at the injection valve.

## *2.2 Waste tubing*

Make the following connections:

- Syringe waste: put the end of the syringe waste tube (see figure 1.1, number 12) in a bottle placed next to the Triathlon.
- Drain wash-position: connect the hose to the drain wash connector of the Triathlon (see figure 1.1, number 7); place the other end of the hose in a bottle placed on the floor. Through this drain all liquid dispensed to waste at the back of the tray is removed.
- Condensed water and leakage: connect the hose to the drain port of the Triathlon (see figure 1.1, number 8). Place the other end of the hose in a waste container on the floor. Through this hose all leakage solvents and condensed water (if Peltier cooling is installed) are drained.

Make sure that the flow path of the hoses is not obstructed in any way.

## *2.3 Wash solvent*

The Triathlon has a 250 mL wash solvent bottle. Execute the following steps to install the 250 mL wash solvent bottle:

1. Fill the wash solvent bottle with the appropriate wash solvent. Use of methanol (organic solvent, no buffers) or a mixture of water and isopropanol (80/20) is recommended. Before using the wash solvent, degas it with Helium or an ultrasonic bath.
2. Screw the bottle to the cap in the holder.
3. Place the holder in the Triathlon as indicated in figure 2.1.
4. Put the wash solvent tube in the wash solvent.
5. Lower the cover of the Triathlon.
6. Select soft function key <SYR END> in the Ready Menu to fill the syringe.
7. Place the syringe back in home position by selecting soft function key <SYR HOME>.

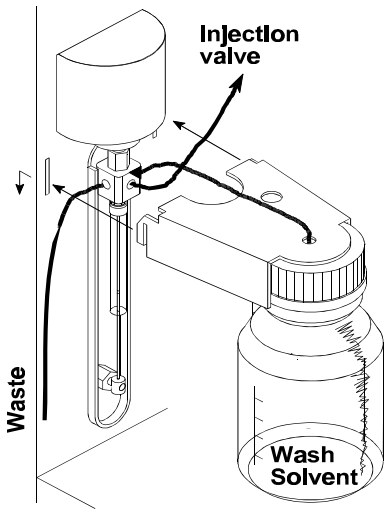



*Figure 2.1 Wash solvent bottle*

8. Repeat steps 6 and 7 until the wash solvent tube and the syringe are completely filled.
9. Select soft function key <WASH> to perform a standard wash routine.
10. If any air remains in the syringe, select <SYR END> again to fill the syringe with wash solvent; press <SYR HOME> again to move contents to waste. Repeat if there is still air in the syringe and gently tap the syringe as wash solvent is dispensed to waste.

If you use an application that requires more than 250 mL of wash solvent for a complete run, install a longer tube (with flanged end for valve fitting) and place a larger bottle next to the Triathlon. To fill the wash solvent tube, you may have to repeat the above-mentioned filling procedure (steps 6 and 7) a few times.

## *2.4 Syringe*

The Triathlon is supplied with a 250 µL syringe. It is also possible to use the Triathlon with a 100µL , 500 µL , 1000 µL or 10 mL syringe. Execute the following steps to install a syringe:

1. Select soft function key <SYR END> in the Ready Menu to move the syringe to end position.
2. Lift the cover.
3. Unscrew the top of the syringe (turn clockwise).
4. Pull the bottom of the syringe towards you; you can now remove the syringe (refer to figure 2.2).
5. Fill the new syringe with wash solvent and make sure that all air bubbles are removed from the syringe.
6. Connect the bottom of the filled syringe to the Triathlon.
7. Screw the top of the filled syringe to the Triathlon (counter clockwise).
8. Lower the cover.
9. Select soft function key <SYR HOME> to remove air from the syringe. The syringe moves to home position and its contents is dispensed to waste.
10. If any air remains in the syringe, select <SYR END> again to fill the syringe with wash solvent; press <SYR HOME> again to move contents to waste. Repeat if there is still air in the syringe and gently tap the syringe as wash solvent is dispensed to waste.
11. Select soft function key <WASH> in the Ready Menu to execute a standard wash routine. All tubing connected to the syringe valve is filled and rinsed.

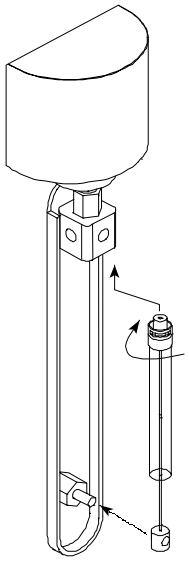


*Figure 2.2 Replacing the syringe*

Select the appropriate syringe in the System Menu and install the correct buffer tubing (refer to section 2.7).

## 2.5 Needle assembly

The needle used for sampling consists of two parts:

- prepuncturing needle: a hollow needle used for puncturing of the septum, capmat or sealer; also used to put headspace pressure on the sample (approximately 0.5 bar).
- sample needle: placed inside the hollow prepuncturing needle; used for the actual transport of sample. Different types of needles can be used here (refer to section 2.6, and options mentioned in Appendix D). If a needle with deviating diameter is used, a different air outlet nut (see figure 2.3, number 6) must be used that matches the injection needle.

Most commercially available sealers or capmats cannot be used in combination with headspace pressure. You are advised to switch off headspace pressure in those cases (System Menu, General Menu).

The following elements can be identified in the needle assembly:

1. Rheodyne Rheflex nut and ferrule
2. Needle tubing
3. Needle connection nut
4. Standard sample needle
5. Needle holder
6. Air outlet nut
7. Prepuncturing needle
8. Sensor (detects tray height, missing tray and missing vials).

Numbers 1, 2, 3, 4, and 6 constitute the sample needle.

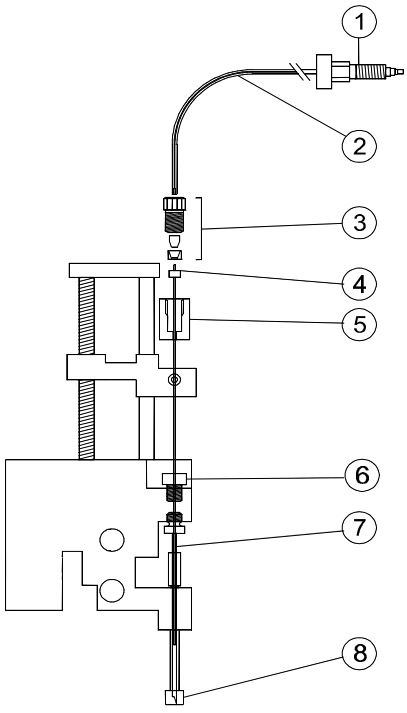



*Figure 2.3 Needle assembly*

Execute the following steps to replace a needle:

1. Loosen the needle connection nut (3).
2. Loosen the Rheodyne Rheflex and ferrule nut (1) .
3. Carefully pull out sample needle and tubing.
4. Insert a new sample needle and tube through the needle holder (5) and tighten the nut (4).
5. Connect the other end of the tube to port 4 of the Rheodyne injection valve using a Rheodyne nut and ferrule. Do not tighten too much to prevent block of tubing.
6. Lower the cover of the Triathlon.
7. Check sample needle height (default height: 2 mm). If necessary, adjust the value in the System Menu (General Menu; refer to Chapter 5).
8. Select soft function key <WASH> in the Ready Menu to clean the new sample needle.

## 2.6 Optional Needles

Needles of the following types can be installed in the Triathlon:

- LSV needle — sample needle with large inner diameter for viscous samples, or in case large volumes are loaded in the loop
- PEEK needle — biocompatible needle
- Fused silica needle — needle with small inner diameter for small injection volumes
- Extended needle — needle for switching valve placed in the side panel of the Triathlon
- Silica-coated needle — needle for use with liquids that would be damaging to a stainless steel needle

Refer to Appendix D for an overview of options available for the Triathlon.

Execute the following steps to install any of these needles:

1. Remove needle as described in section 2.5.
2. Remove the standard air outlet nut (figure 2.3, number 6) and replace it by the nut supplied with the optional sample needle.
3. Install optional sample needle as described in section 2.5.
4. Adjust settings in System Menu (General Menu) to the volume of the new needle tubing (see section 5.2).

## 2.7 Combination of syringe, sample loop and buffer tubing

The 250 µL syringe is the standard syringe; combined with the standard 500 µL buffer and the standard 100 µL sample loop, the following injection volume range is available for the various injection modes:

| | |
|---|---|
| Flushed loop | 100 µL |
| Partial loopfill | 1 - 50 µL |
| µL pick-up | 1 - 27 µL |

The maximum injection volumes are calculated with the following formulas:

| | | |
|---|---|---|
| Flushed loop: | injection volume | = loop volume |
| Partial loopfill: | max. injection volume | = 50% of loop volume |
| µL pick-up: | max. injection volume | = (loop volume - 3 x needle tubing volume) / 2 |

Five sizes of syringes can be used in the Triathlon: 100, 250, 500, 1000 µL and 10 mL. The 10 mL syringe can only be used in the User Program (must be enabled in System Menu; see section 5.3) and not for the standard injection modes.

Flushed loop gives maximum reproducibility (RSD < 0.3%), but not maximum accuracy, since loop volume is specified with an accuracy of ± 10%.
Minimum sample loss = 230 µL (2 x loop overfill + flush volume for needle) for the standard 100 µL loop.

Partial loopfill gives maximum accuracy (depends on syringe accuracy) and reproducibility better than 0.5% RSD for injection volumes > 5 µL
Minimum sample loss (Flush volume) = 30 µL
30 µL is the recommended minimum flush volume (combined with an air segment); smaller flush volumes can be programmed, but will result in decreased performance.

µL Pick-up means zero sample loss, maximum accuracy (same as partial loopfill), but slightly diminished reproducibility: RSD better than 1% for injection volumes > 5 µL.
5 µL of air is injected together with the sample, if an air segment is selected in the System Menu.

For some cases other combinations of syringe, loop and/or buffer are advised:

**Injection volumes smaller than 5 µL:**

- Partial loopfill: use a 100 µL syringe for maximum reproducibility and accuracy. Use a 20 µL sample loop to avoid loss of accuracy due to expansion of the loop content when switching from inject to load position prior to sample loading. Specially when working with high pressure (200 bar), this loss may be 0.1 - 0.5 µL for a 100 µL loop.
  Note that the minimum sample loss in partial loopfill mode is 30 µL (recommended minimum flush volume) for the first injection and an additional 15 (always half the programmed flush volume) for additional injections from the same vial. If a wash between injections has been programmed, sample loss is 30 µL for every injection. For zero sample loss injections, use the µL-pick injection mode.

- µL Pick-up: use a 100 µL syringe for optimum accuracy and reproducibility. Do not use a smaller sample loop! The sample plug is transported into the loop, preceded by a programmable air segment of 5 µL (if selected in System Menu; you are advised to switch off the air segment with µL pick-up), with a plug of transport liquid which equals 2.5 times the programmed needle tubing volume.

**Injection volumes up to twice the standard:**
With the standard 250 µL syringe, standard needle with tubing (15 µL) and standard 500 µL buffer, but with a 200 µL sample loop, the maximum injection volumes are:

| | |
|---|---|
| Flushed loop | 200 µL (sample loss remains 230 µL since loops > 100 µL need only one loop volume overfill; 30 µL pre-flush) |
| Partial loopfill | 100 µL |
| µL Pick-up | 77 µL |

Volumes smaller than 5 µL may be injected, but reproducibility and accuracy may not be < 0.5% for partial loopfill or < 1% for µL pick-up.
In short: loop < 100 µL: loop has to be filled three times
loop ≥ 100 µL - 499 µL: loop has to be filled twice
loop ≥ 500 µL: loop has to be filled 1.5 times

**For volumes larger than 200 µL:**
Use the 2000 µL buffer, use the appropriate sample loop size and the appropriate syringe: Syringe volume > 2 x injection volume. Injection volumes larger than 500 µL are possible, but the sample may contaminate the syringe. Program sufficient wash after use!

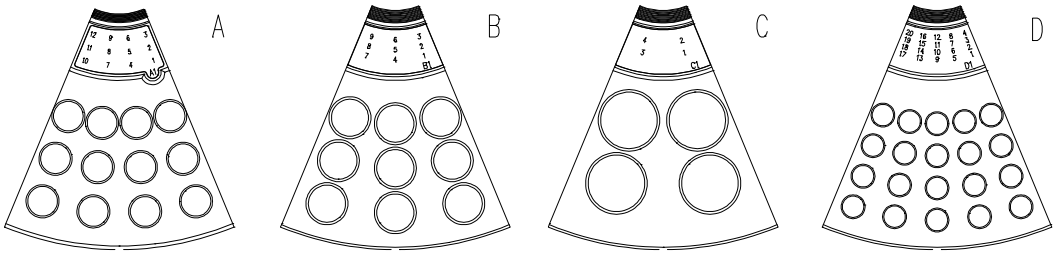
Overview of appropriate buffer tubing for each type of syringe:

| Syringe | Buffer tubing |
|---|---|
| 100 µL | 500 µL |
| 250 µL | 500 µL |
| 500 µL | 2000 µL |
| 1000 µL | 2000 µL |
| 10 mL | 15 mL |

The 10 mL syringe and the 15 mL buffer tubing can only be used in combination with the User Program and not with the standard injection methods.

## *2.8 Tray segments and Vial types*

A wide range of vials and septa can be used for the Triathlon, from micro vials (0.5 mL) to super LSV vials (10 mL). The volumes of standard vials can be reduced by using inserts. The Triathlon can be equipped with four types of tray segments to accomodate use of the various types of vials (see figure 2.4).




Type A: Standard tray segment. Type B: LSV tray segment. Type C: Super LSV tray segment. Type D: Micro vial tray segment.

*Figure 2.4 Types of tray segments*

The Triathlon allows you to use more than one type of segment in a tray. Enter the configuration of the tray in the System Menu (Tray Menu).

The following vial types can be used:

- Chromacol vials for standard tray (outer vial diameter :12mm)

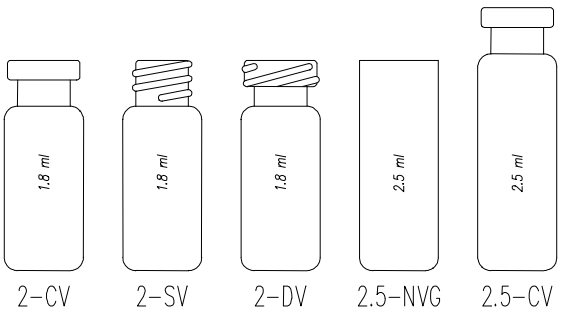



*Figure 2.5 Chromacol vials*

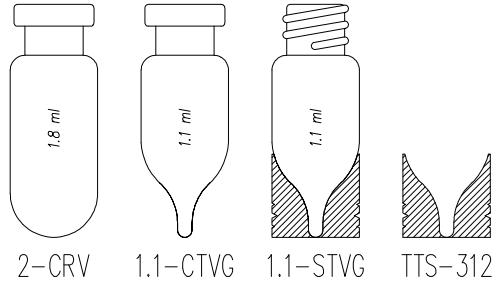



*Figure 2.6 Conical vials with support*

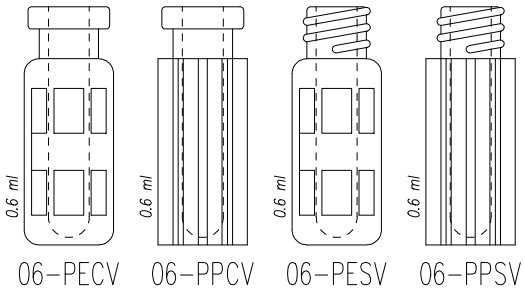



*Figure 2.7 Plastic vials*

Chromacol inserts (02.MTV, 02-MTVWG, 03-MTV) can be used in combination with the appropriate vial and support sleeve or spring.

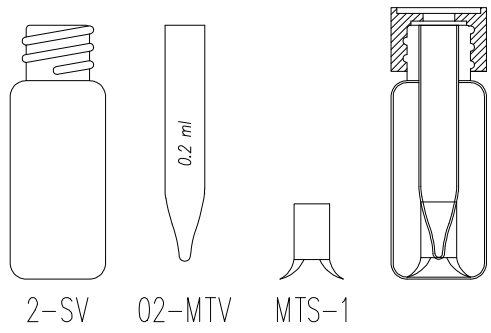



*Figure 2.8 Standard vials with insert*

- Eppendorf micro centrifuge tubes for standard tray

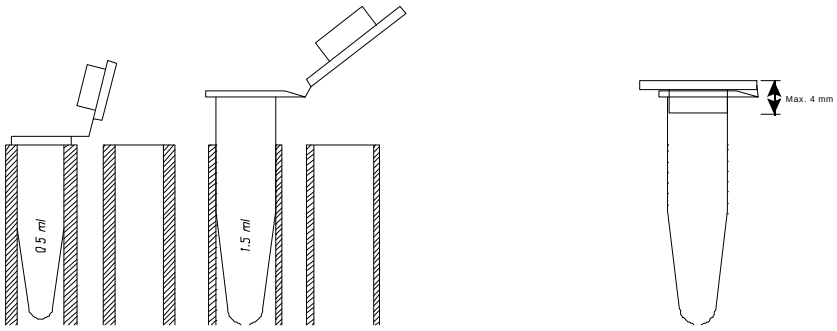



*Figure 2.9 Eppendorf micro centrufuge tubes with support sleeve*

Eppendorf micro centrifuge tubes can be used with a support sleeve. However, the prepuncuring needle may not be able to pierce the caps of some brands of tubes because of the depth of the caps. This may result in damage to the sample needle.

- Chromacol vials for LSV tray (outer vial diameter: 15 mm)

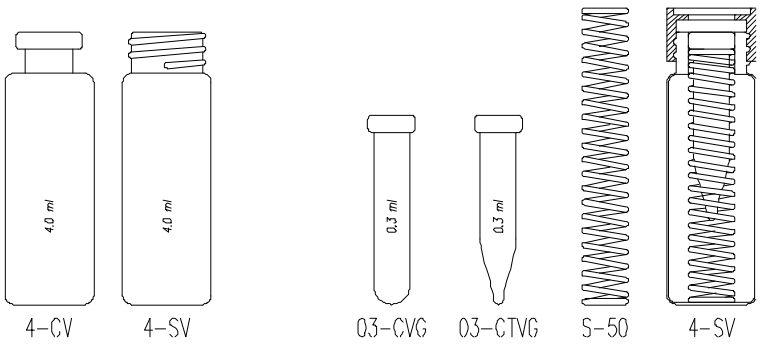



*Figure 2.10 Vials and inserts*

- Chromacol vials for super-LSV tray (outer vial diameter: 22 mm)

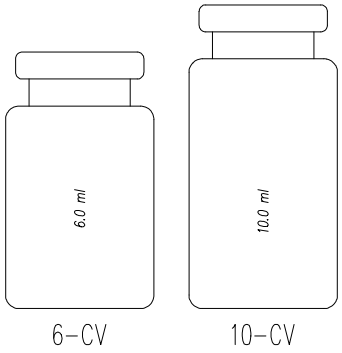



*Figure 2.11 Vials for super-LSV tray*

- Chromacol vials for micro tray (outer vial diameter: 7mm)

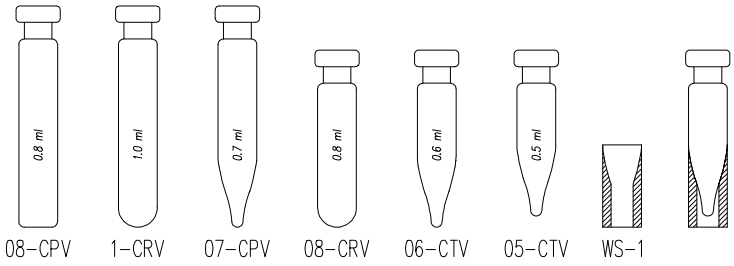



*Figure 2.12 Vials and support*

## *2.9 Vial handling*

It is best to fill vials with a narrow-end pipette to allow air to escape when filling the vial. Do not fill vials to the edge to avoid that sample liquid will be forced into the air needle (risking cross-contamination of samples and fouling of the needles).

If you want to use headspace pressure for a sample handling routine, it is important to make sure that the seal is airtight. An airtight seal is also important to prevent air bubbles from forming and to prevent evaporation of volatile samples.
Check the seal after crimping; if the cap can be turned easily, the seal is not airtight and you will have to adjust the handcrimper.

## *2.10 Loading the sample tray*

The tray segments can be placed in any position in the Triathlon tray. The tray segment types used must be defined in the System Menu (Tray Menu). Tray segments can only be placed in an open position in the front half of the tray. Select <ADVANCE> in the System Menu to rotate the tray if the Triathlon is not executing a run. You can then place a tray segment in the front half of the tray.

Do not place or remove a tray segment at the rear half of the Triathlon tray, as this may damage the tray sensors.

# *Chapter 3. Getting started*

This chapter describes how to get started with the Triathlon. The following will be explained:

- menus of the Triathlon
- convenient working order
- methods that can be programmed and linked to series
- executing a series.

## *3.1 Menus of the Triathlon*

The software of the Triathlon is menu-driven. The most important menus are:

- **Ready Menu**: this menu appears after the Triathlon has been switched on. It offers very general possibilities for among other things methods management and communication with other apparatus.
- **System Menu**: this menu appears after the **System** key in the keypad has been selected. It offers general possibilities for entering settings for an analytical run. Please note that settings may have been factory-installed to suit particular wishes of the user. In that case no changes will have to be made in the System Menu. You are advised to enable only those facilities that you will need, to ensure that menus are as concise as possible.
- **Methods Menu**: this menu appears after the **Methods** key in the keypad has been selected. The menu allows you to program a method to be used in an analytical run and to assign a number to it.
- **Series Menu**: this menu appears after the **Series** key in the keypad has been selected. The menu allows you to define a series, and to assign a number and a method to it for the analytical run.

Use the following **keys** to go through the menus:

| | |
|---|---|
| **E** | ENTER; to confirm a choice or to select a choice made in a screen, or to step through menu lines |
| cursor keys | to change values in a field or to move to a different field in a screen |
| **Escape** | to return to a previous menu |
| **CL** | to remove a value from a field and enter NONE or DEFAULT |
| soft function keys | labels displayed in bottom line of the screen; to go to submenus |

If [MENU] or [MN] is displayed in the top right hand corner of the screen, you can press the **Menu** key in the keypad to display more possibilities offered by the menu. Refer to section 1.4 for an explanation of all keys in the keypad.

## *3.2 Convenient working order*

After you have determined what type of analytical run you want to perform, the most convenient working order for the Triathlon is:

1. Enter settings in the System Menu. Note that the settings for the Triathlon probably already have been correctly entered (factory-installed).
2. Program a method for the analyses you wish to perform in the Methods Menu.
3. Define a series and link a programmed method to a range of vials in the Series Menu.
4. Execute the series.

Please note that it is allowed to use a different order; however, you should keep in mind that settings entered in the System Menu determine which possibilities appear in the Methods and Series menus. Refer to chapter 5 for more information on specific items in menus and the way they influence the other menus.

## *3.3 Types of Methods and links to Series*

The Triathlon offers the following types of methods for different parts of the sample handling routine:

- **injection** method: contains information on the injection routine, flush volume and analysis time.
- **wash** method: describes a wash volume and when a wash must be executed.
- **mix** method: a pre-injection method in which additional sample handling can be performed (e.g. pre-column derivatization).
- **timebase** method: a post-injection method with which outputs to other devices (e.g. integrator or pump) and switching of the ISS valve are controlled.
- **user program**: offers the possibility to program sequences of all actions that can be executed by the Triathlon in separate steps.

Each programmed method is assigned a number. The Triathlon offers the possibility to store a combination of defined methods in a **template**. A template is also identified by a number.

Methods must be linked to series before they can be used. The following possibilities are offered by the Triathlon:

- You can assign an individual **method** to a series: methods (mix, injection, wash, timebase) can be linked to vials in a series.
- You can assign a **template** to a series: a combination of various programmed methods (mix, injection, wash, timebase) can be defined in a template. The template is linked to a range of vials in a series. In this way all steps in an analytical run are laid down and stored.

- You can assign a **user program** to a series: a possibility to combine all possible steps in the analytical process in one program. The user determines the order of the separate actions the Triathlon has to perform.

## *3.4 Executing a Series*

Execution of a series is only possible if you have programmed a method and defined a series for the samples you wish to analyse. Series are not stored in battery backup and exist only for as long as the Triathlon is switched on.
Execute the following steps:

1. Start programming the series by pressing **Start/Stop**.
2. Enter the number of the first series to perform and the number of the last series to perform.
3. Select <START> to start the actual analytical run. The Triathlon starts to execute the series you have defined.

After the Triathlon has executed the run, the Ready Menu will appear again.
Refer to Chapter 4 for a number of examples illustrating this working order.

It is possible to program series and methods during a run. Press **Series** or **Methods**; the possibilities offered in the menus are identical to those offered when the Triathlon is idle.

If a series or method is changed, the new values become active the next time the Triathlon starts a series. The series currently running are not affected by the changes.

## *3.5 Executing a Series in remote control*

To execute a series from remote control, execute the following steps:

1. Press **Start/Stop**.
2. Enter the number of the first and the last series to be performed.
3. Select <REMOTE> to enter the remote control mode. The Triathlon will now operate as slave of another device and can be controlled with Next injection input and Next vial input. To indicate that remote control is active, an “r” is displayed in the bottom left corner of the display during execution of the series. At the end of the series the message “Series completed via remote control” is displayed.
4. Press **Escap**e to return to the Ready Menu.

Refer to Chapter 6 for more information on remote control.

# *Chapter 4. Using the Triathlon*

This chapter describes a number of examples of actions that can be performed with the Triathlon. Please note that this chapter does not describe all types of actions that can be performed. Try to do these examples to learn to work with the Triathlon.
These examples can be executed after the Triathlon has been installed in accordance with Appendix A and after all items described in Chapter 2 have been correctly set up.

## *Example 1. A 10 µL partial loopfill injection*

After the Triathlon has been switched on, an initialization procedure is executed. In this example it is assumed that a loop of 100 µL, needle tubing of 15 µL, a syringe of 250 µL and a type A tray has been installed. Wait until the Ready Menu appears on the screen, then execute the following steps:

**For system settings:**

| Press keys | Description |
|---|---|
| **System** | to enter the System Menu |
| <GENERAL> **E** | to enter the General Menu |
| [0100] **E** | to define the volume of the installed loop |
| [015] **E** | to define the volume of the needle tubing |
| <250> **E** | to define the volume of the syringe |
| <NORMAL> **E** | to set syringe speed to normal |
| [02] **E** | to set sample needle height to 2 mm |
| <YES> **E** | to enable use of air segment |
| <YES> **E** | to switch headspace pressure on |
| **Escape Escape** | to return to the Ready Menu |

For this example all other settings used will be default.

**To program a method:**

| Press keys | Description |
|---|---|
| **Methods** | to enter the Methods Menu |
| <INJECTION> [01] **E** | program injection method number 1 |
| <PARTIAL> **E** | to select partial loopfill injection method |
| [100] **E** | to define an analysis time of 1 minute |
| [030] **E** | to define a flush volume of 30 µL |
| [1] **E** | to define the number of injections per vial |
| [10] **E** | to set the injection volume at 10 µL |
| **Escape Escape** | to return to the Ready Menu |

**To define the series:**

| Press keys | Description |
|---|---|
| **Series** | to enter the Series Menu |
| [01] **E** | to define the Series number |
| [01] **E** | to define the injection method number |
| **CL E** | to enter <NONE> for wash method |
| [01] **E** | to define location of the first sample vial |
| [01] **E** | to define location of the last sample vial |
| **Escape** | to return to the Ready Menu |

**To run the series:**

Place a sample in position A 1 of the tray.

| Press keys | Description |
|---|---|
| **Start/Stop** | to start the Triathlon |
| [01] **E** | to start at series number 1 |
| [01] **E** | to stop after execution of series number 1 |
| <START> | to start the analytical run |

The Triathlon will now locate vial A 01 and perform a 10 µL partial loopfill injection. The display of the Triathlon will indicate the status (Checking tray, Flushing, Loopfill, Running, Rinse buffer, Running). The display also indicates the number of the defined series (01), the method number (01) and the vial on which the analysis is performed (A 01).
At the end of the defined analysis time the Ready Menu will be displayed again to indicate that the Triathlon is ready for the next analytical run.

## *Example 2. A 3 x 10 µL injection with µL pick-up, wash between injections*

In this example a different injection method is used than in the previous one. For that reason several settings must be adapted in the System Menu.

**For system settings:**

| Press keys | Description |
|---|---|
| **System** | to enter the System Menu |
| <GENERAL> **E** | to enter the General Menu |
| **E** until Air segment appears | to go to the Air segment field |
| <NO> **E** | to switch off air segment |
| **Escape** | to return to the System Menu |
| <TRAY> **E** | to enter the Tray Menu |
| **E** until transport vial appears | to go to transport vials field |
| **<**TYPE_A> | to select type A tray segment |
| [1] **E** | to define position of the first transport vial |
| [1] | to define position of the last transport vial |
| **Escape Escape** | to return to the Ready Menu |

**To program a method:**

| Press keys | Description |
|---|---|
| **Methods** | to enter the Methods Menu |
| <INJECTION> [02] **E** | to define method number 02 |
| <PICK-UP> **E** | to select the injection mode for this method |
| [100] **E** | to define the analysis time |
| [3] **E** | to define the number of injections per vial |
| [10] **E** | to define volume of 10 µL for 1st injection |
| [10] **E** | to define volume of 10 µL for 2nd injection |
| [10] **E** | to define volume of 10 µL for 3rd injection |
| **Escape** | to return to the Methods Menu |
| <WASH> | to enter the Wash Menu |
| [01] **E** | to define wash method number 01 |
| <INJECTION> **E** | to select wash between injections |
| [300] | to define the wash volume |
| **Escape Escape** | to return to the Ready Menu |

**To define the series:**

| Press keys | Description |
|---|---|
| **Series** | to enter the Series Menu |
| [01] **E** | to define the series number |
| [02] **E** | to define the injection method for this series |
| [01] **E** | to define the wash method for this series |
| [02] **E** | to define the location of the first sample vial |
| [02] | to define the location of the last sample vial |
| **Escape** | to return to Ready Menu |

**To run the series:**

Put a vial with transport solvent (mobile phase) in transport vial position A 1 and a vial filled with sample at position A 2. Make sure the transport vial is correctly filled before starting a new series.

| Press keys | Description |
|---|---|
| **Start/Stop** | to start the Triathlon |
| [01] **E** | to start at series 01 |
| [01] **E** | to stop after series 01 |
| <START> | to start execution of the series. |

At the end of the defined analysis time the Ready Menu will appear again to indicate that the Triathlon is ready for the following next run.

## *Example 3. A 1:10 dilution followed by a 10 µL partial loopfill injection*

This example describes how to let the Triathlon transfer 360 µL from Reagent A to the destination vial, add 40 µL of sample, mix 3 times with 250 µL and subsequently inject 10 µL.

**For system settings:**

| Press keys | Description |
|---|---|
| **System** | to enter the System Menu |
| <USAGE> **E** | to enter the Usage Menu |
| **E E** | to go to the Mix field |
| <ENABLED> | to enable use of mix methods |
| **Escape Escape** | to return to the Ready Menu |

As soon as a change has been entered in the System settings, the message “ALL SERIES DEFAULT” appears. The user will have to redefine series because the settings have been changed.

**To program the injection method:**

| Press keys | Description |
|---|---|
| **Methods** | to enter the Methods Menu |
| <INJECTION> [03] **E** | to enter the Injection Menu |
| <PARTIAL> **E** | to select partial loopfill injection mode |
| [100] **E** | to define the analysis time |
| [50] **E** | to define the flush volume |
| [3] **E** | to define the number of injections per vial |
| [10] **E** | to enter the injection volume for 1st injection |
| [10] **E** | to enter the injection volume for 2nd injection |
| [10] **E** | to enter the injection volume for 3rd injection |
| **Escape** | to return to the Methods Menu |

**To program the mix method:**

| Press keys | Description |
|---|---|
| <MIX> | to enter the Mix Menu |
| [1] **E** | to define Mix method number 1 |
| <INSERT> | to define mix method step number 1 |
| <ASPIRATE> [5] <AIR> **E** | to aspirate an air segment of 5 µL |
| <INSERT | to define mix method step number 2 |
| <ASPIRATE> [225] | to aspirate 225 µL |
| **Menu** <REAG-A> **E** | to perform defined asp. From reagent vial A |
| <INSERT> | to define mix method step number 3 |
| <DISPENSE> [180] | to dispense 180 µL to |
| ▸ ▸ [02] **E** | destination vial |
| <INSERT> | to define mix method step number 4 |
| <DISPENSE> [50] | to dispense 50 µL |
| <WASTE> ▸ [5] **E** | to dispense defined volume to waste |
| <INSERT> | to define mix method step number 5 |
| <REPEAT> [1] ◂ [4] **E** | to repeat last four steps once |
| <INSERT> | to define mix method step number 6 |
| <ASPIRATE> [5] <AIR> **E** | to aspirate an air segment of 5 µL |
| <INSERT> | to define mix method step number 7 |
| <ASPIRATE> [60] <SAMPLE> **E** | to aspirate 60 µL of |
| <INSERT> | sample |
| <DISPENSE> [40] | to define mix method step number 8 |
| <DESTINATION> ▸ ▸ [2] **E** | to dispense 40 µL |
| <INSERT> | to destination vial |
| <DISPENSE> [50] <WASTE> | to define mix method step number 9 |
| ▸ [5] **E** | to dispense 50 µL |
| <INSERT> | to waste |
| <ASPIRATE> [50] <AIR> **E** | to define mix method step number 10 |
| <INSERT> | to aspirate an air segment of 50 µL |
| <ASPIRATE> [200] | to define mix method step number 11 |
| <DESTINATION> ▸ [3] **E** | to aspirate 200 µL |
| <INSERT> | from the destination vial |
| <DISPENSE> [200] ▸ [9] **E** | to define mix method step number 12 |
| <INSERT> | to dispense 200 µL to the destination vial |
| <REPEAT [3] | to define mix method step number 13 |
| **Escape Escape** | to repeat the last 2 steps three times |
| | to return to the Ready Menu |

**To define the series:**

| Press keys | Description |
|---|---|
| **Series** | to enter the Series Menu |
| [01] **E** | to define series number 1 |
| [01] **E** | to select Mix method number 1 for this series |
| [03] **E** | to select Injection method number 3 |
| **CL E** | to select <NONE> for wash method |
| [1] **E** | to define location of first sample vial |
| [1] **E** | to define location of last sample vial |
| [2] **E** | to define location of first destination vial |
| [3] **E** | to define position of Reagent A |
| **Escape** | to return to the Ready Menu |

**To run the series:**
Put sample in position A 01; position A 02 is used as empty destination vial. Place a filled reagent vial in position A 03. Make sure the reagent vial is filled correctly before starting a new series.

| Press keys | Description |
|---|---|
| **Start/Stop** | To start the Triathlon |
| [01] **E** | To start at series number 1 |
| [01] **E** | To stop after series number 1 |
| <START> | To start processing of sample |

The Triathlon will now start searching for the Reagent vial and transport 180 µL to the destination vial twice, then 40 µL of sample will be added and after mixing 3 times a 10 µL injection will be performed.

## *Example 4. Defining a template and adding a protection code*

This examples describes how to incorporate the injection method (02) and wash method (01) defined in example 2 in a template. A protection code will be added.

**For system settings:**

| Press keys | Description |
|---|---|
| **System** | To enter the System Menu |
| <USAGE> **E** | To enter the Usage Menu |
| [123456] **E** | To enter a 6-digit code (memorize this code!) |

| | |
|---|---|
| **E** | to go to the mix methods field |
| <DISABLED> **E** | to disable use of mix methods |
| **E E** | to go to the template field |
| <ENABLED> | to enable templates |
| **Escape Escape** | to return to the Ready Menu |

After use of templates has been enabled the message "ALL SERIES DEFAULT" appears. The user will have to redefine series because the settings have been changed.

**To select the methods to be incorporated in the template:**

| Press keys | Description |
|---|---|
| **Methods** | to enter the Methods Menu |
| [123456] **E** | to enter the methods protection code |
| <TEMPLATE> | to enter the Template Menu |
| [01] **E** | to define the number for the template |
| [02] **E** | to define the injection method for this template |
| [01] | to define the wash method for this template |
| **Escape Escape** | to return to the Ready Menu |

**To define the series:**

| Press keys | Description |
|---|---|
| **Series** | to enter the Series Menu |
| [01] **E** | to define the Series number |
| [01] **E** | to define the Template method number |
| [01] **E** | to define the first sample vial |
| [02] **E** | to define the last sample vial |
| **Escape** | to return to the Ready Menu |

**To run the series:**

| Press keys | Description |
|---|---|
| **Start/Stop** | to start the Triathlon |
| [01] **E** | to start analysis at series 01 |
| [01] **E** | to stop after analysis of series 01 |
| <START> | to start the analytical run |

The Triathlon now performs the same actions as in Example 2, except that analysis is performed on two vials: A 01 and B 01.
**Note**: select <DEFAULT ALL> in the Ready Menu (Utilities Menu) to erase all series and methods defined in these examples and to default all settings.

# Chapter 5. Reference

This chapter describes all possibilities offered by the Triathlon software, in the order in which they appear in the screen.

## 5.1 Ready Menu

The Ready Menu contains the following soft function keys:

**<ADVANCE>**
Use this key to rotate the tray of the Triathlon. You can now fill the tray with segments by placing the segment at a free position in the front half of the tray.

**<WASH>**
Use this key to start a standard wash procedure. All tubing connected to the syringe valve will be filled and rinsed with wash solvent.

**<SYR END>**
Use this key to move the syringe to end position if you wish to replace the syringe needle or to simplify filling of wash solvent tubing. A syringe volume of wash solvent is aspirated from the wash solvent bottle and the wash solvent tube is filled.
Select soft function key <SYR HOME> to dispense the syringe contents to syringe waste and to move the syringe to standard operating position again.

**<UTILS>**
Use this key to go to the Utilities Menu. If use of a method protection code is enabled in the System Menu, the code must be entered to access the Utilities Menu. The menu offers the following possibilities:

**<COPY>** to copy a method. Enter the type (mix, injection, timebase, wash) and the number of the method to be copied. Then enter a number to define the destination method. Any existing method stored under that number will be overwritten.

**<ERASE>** to erase a method (Template, Methods, User Program). If Template and User Program are disabled in the System Menu, the soft function keys for erasing a standard Method (mix, injection, wash, timebase) appear. Note that it is not possible to erase the user program if the protection code for the user program is enabled in the System Menu.

**<LOG>** the Triathlon keeps a log of system-relevant events (<EVENTS>; records error messages that have been generated) and keeps count of actions of valves and syringe movements (<COUNT>). A message appears after every 50,000 syringe actions and after every 200,000 syringe valve actions: “Lifetime of syringe (valve) maybe exceeded. Check for possible leakage!” .
**Syringe**: if you do not replace the syringe at this moment and tell the system “not to display this message again”, the message will not be displayed again until 50,000 more syringe actions have been counted.
**Syringe valve**: will have to be replaced and the counter for valve actions will have to be reset by the maintenance engineer.

**<DEFAULT ALL>** to change all software settings to default. All series, methods, templates and the user program (unless protected by protection code) will be erased.

If <DEFAULT ALL> is selected, check whether hardware configuration still is compatible with settings entered in the System Menu.

**<SSV>** (option)
Use this key to start a procedure in which all lines of the solvent selection valve can be primed. The menu offers the following possibilities:

**<SSV1>** to **<SSV6>** to open the corresponding port of the solvent selection valve.

**<PRIME>** to prime the selected solvent line with one syringe volume.The last selected port will remain active after leaving the SSV mode.

**<COOL>** (option)
Use this key to enter the programming mode for Peltier tray cooling. The programmable temperature range is 4°C to 40°C. The maximum cooling capacity is approximately 20°C below ambient (refer to Appendix B for specifications). Connect the condensed water and leakage connector to a waste container on the floor to drain condensed water.
If the cool option is switched <ON> the following soft function keys can be selected:

**<MANUAL>** temperature control will remain OFF until it is switched on again by the user (in this menu).

**<AUTOMATIC>** temperature control will be switched OFF after all programmed series have been executed.

**<DATE-TIME>** temperature control will be switched OFF at a date and time that can be programmed.

**<SERIAL>**
Use this key to put the Triathlon in serial mode to allow for control of the autosampler by way of PC (RS232 interface). Select a device identifier in the System Menu (refer to section 5.2). If a method protection code was defined in the system settings, this code must be entered to get access to serial mode. The following soft function keys appear:

| | |
|---|---|
| **<PANIC>** | press this key to begin a stop sequence in which all tubing is rinsed and the valve and I/O ports are reset. At the end of the sequence serial mode is resumed. |
| **<EXIT>** | press this key to end serial mode and return to the Ready Menu. |

**<SERVICE>**
For service to the apparatus. To be used by authorized personnel only. The Service Menu is protected by a service code.

## *5.2 System Menu*

The System Menu contains the following soft function keys:

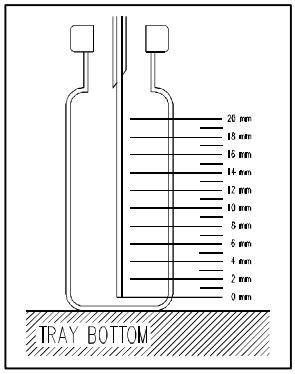
**<GENERAL>**
Press this key to enter values for:

- **loop volume, needle tubing, syringe volume**: these values have to be entered because the Triathlon can be fitted with various types of syringes. Every needle and needle tubing volume requires a different minimum flush volume. The default flush volume equals two times the volume of needle and tubing.

  The 10 mL syringe can only be selected if the use of the User Program is enabled in the System Menu (Usage Menu). When the 10 mL syringe is selected, only the User Program can be used. Use of other types of methods will be disabled.

- **syringe speed and scale factor:** The aspirating speed of the syringe used in injection methods can be adapted depending on viscosity of samples. Alternatively syringe speeds can be reduced by entering a scale factor. The syringe speed will be the scale factor multiplied by the syringe speed. The speed of the syringe during the wash or the rinsing procedure of the buffer are not affected by this setting. (Refer to table 5.3, page 42).

- **needle height:** distance between the needle point and a reference point, a few millimetres above the bottom of the sample tray(**Not** the bottom of the vial, see figure 5.1), can be programmed. The value in the system settings is only used in injection methods, for mix methods this value is programmable in the method itself).



A height of 0 mm is not the bottom of the tray, but a reference value related to a point a 2.5 mm above the bottom of the tray. Check sample needle height with an empty vial.

- **skip missing vials**: YES means that empty spaces are skipped during the run. NO means that the Triathlon will stop if an empty space is encountered during the run; an error code will be generated.

- **air segment**: decide whether an air segment will be used for analytical runs (for explanation of air segment refer to Appendix H).

.

- **headspace pressure**: to switch headspace pressure on or off. The Triathlon uses headspace pressure to facilitate transport of sample into the loop. The compressor will always be used during a wash procedure. Please note that accuracy and reproducibility may decrease if headspace pressure is switched off. However, headspace pressure will only be useful if sample vials are airtight (refer to sections 2.5 and 2.8).

- **time display**: offers a choice between two types of time representation.

- **key click, error beep and alarm buzzer**: offer the possibility to switch sound signals on or off.

*Table 5.1 Overview of General settings*

| general setting for | default | possible range |
|---|---|---|
| loop volume | 100 µL | 5 - 1000 µL |
| needle tubing | 15 µL | 1 - 200 µL |
| syringe volume | 250 µL | 100, 250, 500, 1000 µL or 10 mL |
| syringe speed | normal | low, normal or high |
| scale factor | 1.0 | 0.1 - 1.0 |
| needle height | 2 mm | 0 - 40 mm |
| skip missing vials | yes | yes or no |
| air segment | yes | yes or no |
| headspace pressure | yes | yes or no |
| time base display | HH:MM:SS | H:MM:SS or H:MM:mm |
| key click | on | on or off |
| error beep | on | on or off |
| alarm buzzer | on | on or off |

## <USAGE>

Press this key to enter the following Usage settings:

- **protection code**: to enter a code for protection of methods. Enter a six digit code (000000-999999) for protection of all methods. Press **CL** to erase the code. If a code has been defined it is not possible to enter the System Menu and the programming menus without entering the protection code. Default: none.

- **timebase methods**: to enable or disable the possibility to program timebase methods. The Triathlon controls other connected equipment during analysis time. Program timebase methods in the Methods Menu. Default: disabled.

- **mix methods**: to enable or disable the possibility to program mix methods for the Triathlon. Program mix methods in the Methods Menu. Please note that the Triathlon cannot analyze priority samples during a run if the mix method is enabled. Default: disabled.

- **user program**: to enable or disable the possibility to program a user program. If this function is enabled it is possible to enter a user program protection code (6 digits). Program the user program in the Methods Menu. Please note that the Triathlon cannot analyze priority samples during a run if the user program is enabled.
  Default: disabled.

- **labeled vials**: to enable or disable the possibility to program labeled vials. Program the location of labelled vials in the Series Menu. Default: disabled.

- **templates**: to enable or disable the possibility to program templates. Pogram templates in the Methods Menu. Default: disabled.

- **calibration vials**: to enable or disable the possibility of programming calibration vials. Program the location of the calibration vials in the Series Menu. Default: disabled.

You are advised to disable as many functions in the Usage Menu as possible to make sure that other menus do not contain possibilities that are irrelevant for the type of analyses you are to perform.

### <TRAY>

Use this key to define the type of tray that will be used. Four types of tray segments can be selected: Type A (default), type B, type C and type D numbered 1-8 (see fig. 2.4). After all tray types have been has been selected, enter:

- **type of tranport vials (A-D),** or press **CL**
- **first transport vial**: enter a number
- **last transport vial**: enter a number

Vials can be placed in any of the vial positions. Transport vials must be placed in a continuous row.

### <IO>

Use this key to enter the I/O configuration mode and define the following:

- **vial output BCD or HEX**: to define the vial output.
- **inject-marker pulse length**: to define the length of the inject-marker pulse.
- **vial-marker pulse length**: to define the length of the vial-marker pulse.
- **labeled vial marker pulse length**: to define the length of the vial-marker pulse of the labeled vial.
- **input edge next injection**: to define the edge sensitive inputs for the next injection.
- **input edge next vial**: to define the edge sensitive inputs for the next vial.
- **freeze input active**: to define whether the freeze input is active when high, or freeze input is active when low.
- **reset outputs after last series**: to determine whether the outputs should be reset to default after the last series.

Refer to Chapter 6 for more specific information on I/O connections.

*Table 5.2 Overview I/O menu*

| Possibility | default | Range |
|---|---|---|
| inject-marker pulse length | 1.0 s | 0.1 - 2.0 |
| vial-marker pulse length | 1.0 s | 0.1 - 2.0 |
| labeled vial marker pulse length | 1.0 s | 0.1 - 2.0 |
| input edge next injection | falling | falling or rising |
| input edge next vial | falling | falling or rising |
| freeze input active | low | low or high |
| reset outputs after last series | no | yes or no |

### <CLOCK>

Use this key to switch the system clock on or off. Select ON to enter the clock menu in which you can set date (yy,mm,dd) and time (hh,mm). This date and time will be displayed in the Ready Menu.

### <COMM.>

Use this key to define a device identifier for communication with other equipment (e.g. a PC). An identifier between 20 and 29 can be selected for the Triathlon.

## *5.3 Methods Menu*

This menu allows the user to program various types of methods: it is possible to define 24 separate injection methods, 5 wash methods, 5 timebase methods, 9 mix methods and one user program.
It is also possible to program a combination of methods and save them in a **template**. The settings entered in the System Menu determine the possibilities offered by the Methods Menu.

### <TEMPLATE>

Use this key to enter a menu in which the contents of a template can be defined. First assign a number to the template, then link the numbers of methods to the template. The following items can be entered to fill a template:

- **user program instead of methods**: if soft function key <YES> is selected, the complete template is filled with the user program; no other methods can be added. If soft function key <NO> is selected the template can be filled with the following:
- **mix method number**
  **injection method number**
  **wash method number**
  **timebase method number.**

A maximum of 24 templates can be programmed.

## <METHODS>

Use this key to enter a menu in which methods can be defined:

**<MIX>** (if enabled in System Menu, Usage Menu)
Use this key to program a method that allows you to perform pre-injection sample handling, e.g. pre-column derivatization, dilution or adding of internal standard. Nine mix methods can be programmed; the maximum number of steps that can be programmed for the total of 9 mix methods and the user program is 240. Assign a number to the mix method. The Mix Menu appears:

- <EDIT> an existing step or a new step for a new mix method
- <INSERT> a new step in an existing method before the displayed step
- <DELETE> the displayed step.

"End of mix method" means that the mix method is empty; if an existing mix method is selected, the first line of the mix method is displayed. Scroll through the steps of the existing method with the cursor keys and use the soft function keys to enter changes in an existing method.
The following types of steps can be programmed for a mix method:

**<ASPIRATE>** (sample, air, destination, reagent A-D) a programmed volume. Speed of syringe can be selected from 1-9. (Refer to table 5.1, page 34 for values). Height (H) indicated is the distance of the needle point to the tray holder (default: 2 mm).The maximum amount which can be aspirated is the total volume of the syringe.

**<DISPENSE>** (sample, waste, destination, reagent A-D) a programmed volume from the buffer tubing. Speed of the syringe can be selected from 1 - 9. (Refer to table 5.1, page 34). Height (H) indicated is the distance of the needle point to the sample tray (default: 2 mm). It is possible to dispense a larger volume than the volume aspirated in previous actions. The aspirated amount will be complemented with liquid from the wash solvent bottle to total the programmed dispense volume.

**<WAIT>** to define a pause (H:MM:SS, maximum of 9 hours, 59 minutes and 59 seconds).

During the pause, the needle will move to home position (if the previous step is an aspirate or dispense action). If you want the needle to stay in the same position, an aspirate or dispense step of 0 µL must be programmed at the desired position.

**<REPEAT>** Enter the number of steps that must be repeated and how often they must be repeated.

**<WASH>** enter the volume for needle wash. Buffer is rinsed to waste.

**<INJECTION>**
Use this key to program a method that defines injection methods (max. 24) for a run of the types full loop, partial loopfill or µL pick-up. Enter a number for the injection method you are going to program. If the selected method is locked because of changes in the settings (System Menu) after programming the method, the word LOCK is displayed. The method can be unlocked by programming valid values in the method itself or by restoring the values in the System Menu.
Use the soft function keys to select an injection method, then enter values for:

- **analysis time**: the time between switching the injection valve to inject and the start of processing the next sample.

- **flush volume**: the amount of sample taken from a vial before the loop is filled with sample. Default value: 30 µL (combined with an air segment).

Flush volumes of less than twice the volume of the needle and tubing will result in decreased performance.

- **number of injections per vial:** maximum value is 9.

- **injection volume:** can be entered for each injection per vial. The maximum programmable injection volumes are:
  **partial loopfill**: 50% of the programmed loop volume
  **µL pick-up**: injection volume = (loop volume – 3 x needle volume)/2
  **flushed loop**: not programmable, is equal to the loop volume but needs more sample to fill the loop (3 x loop volume for loop volumes < 100 µL; 2 x loop volume for loop volumes ≥ 100 µL - 499 µL; 1.5 x loop volume for loop volumes ≥ 500 µL).

**<WASH>**
Use this key to program wash methods. It is possible to program a wash between injections, samples or series. For each wash method the volume of wash solvent can be defined. The minimum programmable volume is 300 µL.

**<TIMEBASE>** (if enabled in System Menu, Usage Menu)
Press this key to enable control of the optional ISS valve and other devices via auxiliary or binary outputs. A maximum of 5 timebase methods can be programmed. The menu offers the following soft function keys:

**<AUX>** scroll through all program lines by pressing **E** or select AUX to move to the next auxiliary.

**<VALVES>** controls the ISS valve and the solvent selection valve. The ISS valve can only be programmed if the optional ISS valve is installed. 6-1 and 2-1 refer to the interconnected ports of the valves. Press **E** to scroll through programming lines (Only if SSV option is installed). Enter the time and the SSV port number (value between 1 and 6).

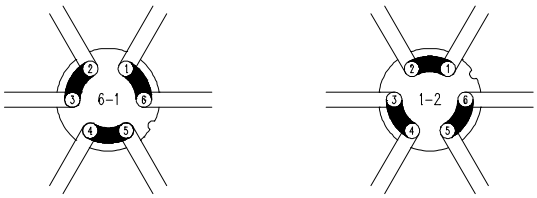



*Figure 5.2 ISS valve; interconnected ports*

**<CODE>** outputs: enter a time and a value between1 and 15, hexadecimal output. Press **E** to scroll through the programming lines.

**<END>** to enter the end time for timed events program; press **E** to scroll through the programming lines. If no value is filled in or if **CL** is pressed, the Triathlon will automatically generate an end time. The end time is equal to the analysis time programmed in the injection method used in the same series.

If end time exceeds the programmed analysis time, this end time overrules the analysis time.
It is possible to program events after the end time, but these events are not carried out during a run.

**<USER PROGRAM>**
The user program offers the possibility to program all possible actions required for a sample handling sequence in separate steps. Note that the total number of steps for the user program and all nine mix methods cannot exceed 240. The user program can be protected by a special user program protection code (System Settings, Usage Menu). If no user program has been programmed yet, “end of user program” is displayed. Otherwise, the first line of the programmed method appears. The soft function keys appear:

- <EDIT> an existing step or program a new step for the user program
- <INSERT> a new step before the displayed step
- <DELETE> delete the displayed step.

The edit and insert menus offer the following soft function keys:

**<ASPIRATE** a programmed volume from sample vial, ambient air, destination vial, wash, or one of the reagent vials into the buffer tubing. Speed and height of syringe can be entered (refer to table 5.1, page 34). The maximum volume that can be aspirated is the total volume of the syringe.

**<DISPENSE>** a programmed volume from the buffer tubing into the sample vial, waste , destination vial, wash or one of the reagent vials. Speed and height of syringe can be entered (refer to table 5.1, page 34).
It is not possible to dispense a larger volume than the total volume aspirated in previous actions.

**<SYR_VALVE>** to control the connections of the syringe to one of its three tubes:
<NEEDLE>: connection to sample needle
<WASH>: connection to wash solvent bottle
<WASTE>: connection to syringe waste tubing.

**<SYR>** to control the movements of the syringe.
<LOAD>: the syringe with the programmed volume
<UNLOAD>: the syringe with the programmed volume
<HOME>: the volume previously aspirated will be dispensed to the last programmed position, and the syringe will be initialized again.

**<WASH>** to execute a needle wash; the content of the buffer tubing is not rinsed to waste before the start of the wash. The programmed volume of wash solvent is used to wash the needle at the wash position.

The wash position may be contaminated with the contents of the buffer tubing, which may generate cross-contamination. To prevent contamination of the wash position, program a dispense to waste action before programming a wash action.

**<VALVES>** to program positions of high pressure valves (ISS, injector valve, SSV). The injector valve has two positions: <INJECT> and <LOAD>. The ISS optional valve has positions 1-6 and 1-2 (see figure 5.2).

**<WAIT>** to program a pause (max. 9 hours, 59 minutes, 59 seconds).

During the pause, the needle will move to home position (if the previous step is an aspirate or dispense action). If you want the needle to stay in the same position, an aspirate or dispense step of 0 µL must be programmed at the desired position.

**<COMPRES>** to activate the compressor to put air pressure on a sample. The compressor will stay active until it is switched off (in a next programmed step). The compressor will be automatically switched off at the end of the needle wash routine if a needle wash is used.

**<AUX>** to control the four standard auxiliaries (contact closures). Refer to Chapter 6.

**<WAIT-IN>** to program a pause in which the Triathlon waits for one of the four inputs to become <HIGH> or <LOW> before continuing with the next step. Refer to Chapter 6.

**<PROG-OUT>** to define two programmable outputs (contact closures). These are similar to the auxiliaries, but only available in the user program. Refer to Chapter 6.

**<CODE>** to program the output to the connector P3 TIMED OUTPUTS. This is a HEX output in the range 0 to 15. Refer to Chapter 6.

**<MARKERS>** the markers normally generated in the Triathlon are not active in the user program, but can be programmed in this screen (refer to Chapter 6). Select marker and status (inject, vial, labeled).

**<SSV>** (option): to define the Solvent Selection Valve (SSV) port position, range 1 to 6.

*Table 5.3 Syringe speed*

| | SYRINGE | | | | |
|---|---|---|---|---|---|
| **SPEED** | ***100 µL*** | ***250 µL*** | ***500 µL*** | ***1000 µL*** | ***10 mL*** |
| **1** | 50 µL/min | 125 µL/min | 250 µL/min | 500 µL/min | 0.5 mL/min |
| **2 (low)** | 125 µL/min | 315 µL/min | 630 µL/min | 1255 µL/min | 1.3 mL/min |
| **3 (normal)** | 250 µL/min | 625 µL/min | 1250 µL/min | 2495 µL/min | 2.5 mL/min |
| **4 (high)** | 375 µL/min | 940 µL/min | 1880 µL/min | 3765 µL/min | 3.8 mL/min |
| **5** | 770 µL/min | 1920 µL/min | 3840 µL/min | 7680 µL/min | 7.7 mL/min |
| **6** | 1070 µL/min | 2675 µL/min | 5335 µL/min | 10665 µL/min | 10.7 mL/min |
| **7** | 1370 µL/min | 3430 µL/min | 6855 µL/min | 13710 µL/min | 13.7 mL/min |
| **8** | 1745 µL/min | 4365 µL/min | 8725 µL/min | 17450 µL/min | 17.5 mL/min |
| **9** | 2135 µL/min | 5335 µL/min | 10670 µL/min | 21335 µL/min | 21.3 mL/min |

During the dispense action the pressure in the buffer tubing will increase. To prevent damage of the buffer tubing, the flow should not exceed the value of 6 mL/min for water. (Maximum speed 9 for 100 µL and 250 µL-syringes, speed 6 for a 500 µL syringe and speed 4 for 1000 µL and 10 mL syringe.) If more viscous liquids are used the speeds should be reduced.

## 5.4 Series Menu

This menu allows you to define the run sequence in a series. A maximum of 24 series can be programmed. A series contains information about the methods to be used for a range of vials. This can be a template, a separate method (mix, injection, wash, timebase), or the user program. Information on location of vials, labeled vials or calibration vials is also programmed in a series.

The settings entered in the System Menu and the methods defined in the Methods Menu determine which possibilities appear in the Series Menu.

*Table 5.4 Series parameters*

| ***Without templates*** | ***With templates*** |
|---|---|
| ❍ Use user programYes/No<br>● Injection method number<br>● Wash method number<br>❍Time base methods number<br>❍ Mix method number<br>*Time base and mix method are only available if enabled in the System Menu* | ● Template number |
| ❍ Use calibration vials Yes/No<br>❍ First calibration vial<br>❍ Last calibration vial<br>❍ No. of samples between calibration<br>*Calibration vials are only available if enabled in the System Menu; not available if Mix Method has been programmed* | |
| ● First sample vial<br>● Last sample vial | |
| *Only if a mix method has been programmed, or if used in user program:*<br>❍ First destination vial<br>❍ Vial Reagent-A<br>❍ Vial Reagent-B<br>❍ Vial Reagent-C<br>❍ Vial Reagent-D | |
| *Only if the use of labeled vials has been enabled in the System Menu:*<br>❍ Labeled vial no. 1<br>❍ Labeled vial no. 2<br>❍ Labeled vial no. 3<br>❍ Labeled vial no. 4 | |

● *marked questions are always asked in series,*
❍ *marked questions depend on the used methods and the settings entered in the System Menu.*

**Explanation:**
After you have entered the required settings in the System Menu and after you have programmed methods to be used for an analytical run, you can press **Series** to enter the Series Menu. Table 5.4 gives an overview of the items you have to define for the Series.

- With Templates
  If you are going to execute an analytical run by way of a template, you will only be asked to enter the template number and to indicate the location of the first sample vial and the last sample vial.

- Without Templates
  If you are going to execute an analytical run without using a template, you will be asked to enter an injection method number and a wash method number, and you will have to indicate the location of the first sample vial and last sample vial.

If you have enabled use of calibration vials in the System Menu (Usage Menu), you will have to define whether you will use calibration vials, and indicate the location of the first and last calibration vial, and indicate the number of vials between calibration vials (refer to figure 5.3).
However, if you have for example enabled use of a Mix Method in the System Menu (Usage Menu), you will also have to define the location of the First destination vial and Reagent vials.

Series are stored in the Triathlon memory for as long as the power is on. As soon as power is switched off, all programmed series will be deleted.
It is not possible to leave the Series Menu before all values have been programmed.

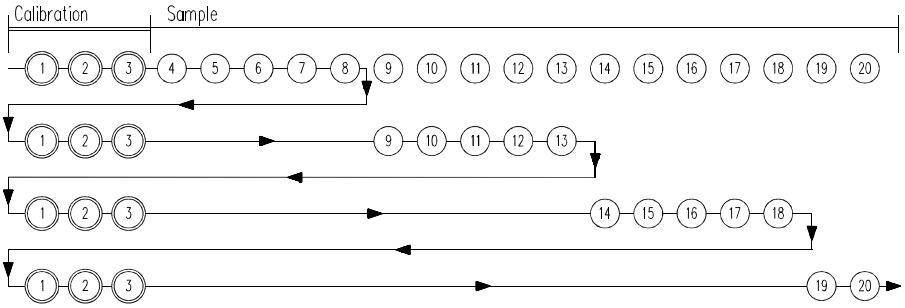



*Figure 5.3 Injection sequence with 3 calibration vials between every 5 vials*

# Chapter 6. I/O Connections

The Triathlon has six standard I/O connectors on the rear side; five OUTPUT connectors and one INPUT connector. Refer to figure 1.2 for location of connectors.

The communication connector is a standard RS232 or an RS422/485 communication interface connector. The configuration of the I/O connector is described in the tables below.

The manufacturer will not accept any liability for damages directly or indirectly caused by connecting the Triathlon to instruments which do not meet relevant safety standards.

## 6.1 Contact closures outputs

The following three tables with programmable outputs (P1), marker outputs (P4) and auxiliary outputs (P5) are contact closures outputs (floating NO/NC contact); see figure 6.1, page 44.

*Table 6.1 Connector P1 OUTPUTS (2 programmable outputs and alarm output)*

| | | | |
|---|---|---|---|
| 1 | OUT 1 - Normally open | 8 | Spare |
| 2 | OUT 1 - Common | 9 | Spare |
| 3 | OUT 1 - Normally closed | 10 | Alarm output - Normally open |
| 4 | OUT 2 - Normally open | 11 | Alarm output - Common |
| 5 | OUT 2 - Common | 12 | Alarm output - Normally closed |
| 6 | OUT 2 - Normally closed | 13 | 24 V DC |
| 7 | Spare | 14 | Power ground |
| | | 15 | Power ground |

$V_{MAX}$ = 28 $V_{DC}$ / $V_{AC}$, $I_{MAX}$ = 0.25 A

The Alarm output will be activated whenever an error occurs; refer to Appendix C for a description of the error codes of the Triathlon.

*Table 6.2 Connector P4 MARKERS*

| | | | |
|---|---|---|---|
| 1 | Inject marker - Normally open | 8 | Labeled vial marker - Common |
| 2 | Inject marker - Common | 9 | Labeled vial marker - Normally closed |
| 3 | Inject marker - Normally closed | 10 | STOP I/O - Normally open |
| 4 | Vial marker - Normally open | 11 | STOP I/O - Common |
| 5 | Vial marker - Common | 12 | STOP I/O - Normally closed |
| 6 | Vial marker - Normally closed | 13 | 24 V DC |
| 7 | Labeled vial marker - Normally open | 14 | Power ground |
| | | 15 | Power ground |

$V_{MAX}$ = 28 $V_{DC}$ / $V_{AC}$, $I_{MAX}$ = 0.25 A

*Table 6.3 Connector P5 AUXILIARIES*

| | | | |
|---|---|---|---|
| 1 | AUX 1 - Normally open | 8 | AUX 3 - Common |
| 2 | AUX 1 - Common | 9 | AUX 3 - Normally closed |
| 3 | AUX 1 - Normally closed | 10 | AUX 4 - Normally open |
| 4 | AUX 2 - Normally open | 11 | AUX 4 - Common |
| 5 | AUX 2 - Common | 12 | AUX 4 - Normally closed |
| 6 | AUX 2 - Normally closed | 13 | 24 V DC |
| 7 | AUX 3 - Normally open | 14 | Power ground |
| | | 15 | Power ground |

$V_{MAX}$ = 28 $V_{DC}$ / $V_{AC}$, $I_{MAX}$ = 0.25 A

Maximum current for 24 $V_{DC}$ supply is 0.5 A total.

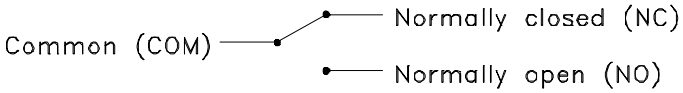



*Figure 6.1 Contact closures outputs*

## 6.2 TTL outputs

The following tables show the marker outputs (P2) and a 4 bit time base code output (P3), programmable in a time base method. Both connectors are TTL level outputs, a connection diagram is shown in figure 6.2 (page 45).

*Table 6.4 Connector P2 TTL OUTPUTS*

| | | | |
|---|---|---|---|
| 1 | INJECT MARKER | 9 | D 4 (BCD or HEX) (10 or 16) |
| 2 | VIAL/VIAL MARKER | 10 | D 5 (BCD or HEX) (20 or 32) |
| 3 | LABELED VIAL MARKER | 11 | D 6 (BCD or HEX) (40 or 64) |
| 4 | STOP I/O | 12 | D 7 (BCD or HEX) (80 or 128) |
| 5 | D O (BCD or HEX) (1) | 13 | Signal ground |
| 6 | D 1 (BCD or HEX) (2) | 14 | Signal ground |
| 7 | D 2 (BCD or HEX) (3) | 15 | Signal ground |
| 8 | D 3 (BCD or HEX) (4) | | |

All markers are active low (logical 0).
$V_{MAX}$ = 5.5 V, logical 1 > 3.5 V , logical 0 < 1.0 V. DC output source / sink current ± 20 mA.

A marker output pulse will be generated when the injection valve switches from LOAD to INJECT. However, in a User Program markers have to be programmed by the user.

**BCD: Binary Coded Decimal**
The BCD output consists of a 2-digit vial number. D3-D0 represent the single digits (0-9) and D7-D4 represent the tens.

*Table 6.5 BCD output conversion*

| Value<br>• = active | Output<br>D 0<br>(1) | <br>D 1<br>(2) | <br>D 2<br>(4) | <br>D 3<br>(8) |
|---|---|---|---|---|
| 0 | | | | |
| 1 | • | | | |
| 2 | | • | | |
| 3 | • | • | | |
| 4 | | | • | |
| 5 | • | | • | |
| 6 | | • | • | |
| 7 | • | • | • | |
| 8 | | | | • |
| 9 | • | | | • |

*Table 6.6 Examples of BCD vial number output*

| **BCD** | Tens | | | | Single digit | | | | Vial number |
|---|---|---|---|---|---|---|---|---|---|
| | D7 | D6 | D5 | D4 | D3 | D2 | D1 | D0 | |
| | (8) | (4) | (2) | (1) | (8) | (4) | (2) | (1) | |
| Output | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 96<br>90 + 6 |
| | 9 x 10 ($10^1$) | | | | 6 x 1 ($10^\circ$) | | | | |
| Output | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 59<br>50 + 9 |
| | 5 x 10 ($10^{1)}$ | | | | 9 x 1 ($10^\circ$) | | | | |
| Output | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 10<br>10 + 0 |
| | 1 x 10 ($10^1$) | | | | 0 x 1 ($10^\circ$) | | | | |

### Hex: Hexadecimal Code

The hexadecimal output represents the vial number as 8 bits by assigning values of 1, 2, 4, 8, 16, 32, 64 and 128 to every single bit (D0-D7).

*Table 6.7 Examples of hexadecimal output*

| **HEX** | D7 (128) | D6 (64) | D5 (32) | D4 (16) | D3 (8) | D2 (4) | D1 (2) | D0 (1) | Vial number |
|---|---|---|---|---|---|---|---|---|---|
| Output | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | **150** |
| | 128 + 16 + 4 + 2 | | | | | | | | |
| Output | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | **96** |
| | 64 + 32 | | | | | | | | |
| Output | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | **85** |
| | 64 + 16 + 4 + 1 | | | | | | | | |
| Output | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | **59** |
| | 32+ 16 + 8 + 2 + 1 | | | | | | | | |

*Table 6.8 Connector P3 TIMED OUTPUTS; 4 bit time base code output*

| | |
|---|---|
| 1 TB 0 (HEX) (1) | 6 Signal ground |
| 2 TB 1 (HEX) (2) | 7 Signal ground |
| 3 TB 2 (HEX) (4) | 8 Signal ground |
| 4 TB 3 (HEX) (8) | 9 Signal ground |
| 5 not used | |

$V_{MAX}$ = 5.5 V, logical 1 > 3.5 V , logical 0 < 1.0 V. DC output source / sink current ± 20 mA.

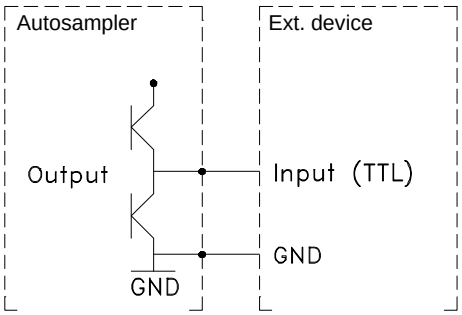



*Figure 6.2 TTL output*

## 6.3 TTL inputs

The following connector is an active high or active low TTL input; it can be defined in the System Menu. The NEXT INJECTION INPUT and the NEXT VIAL INPUT can be used when the Triathlon works in REMOTE CONTROL. The FREEZE INPUT and STOP I/O input can be used to control the Triathlon by other devices. The four inputs (INPUT 1 to 4) can only be used in the user program, e.g. to control the sequence of the steps in this method. A connection diagram is shown in figure 6.3 (page 46).

*Table 6.9 Connector P6 INPUTS (TTL)*

| | | | |
|---|---|---|---|
| 1 | NEXT INJECTION INPUT | 9 | Signal ground |
| 2 | NEXT VIAL INPUT | 10 | Signal ground |
| 3 | FREEZE INPUT | 11 | Signal ground |
| 4 | STOP I/O | 12 | Signal ground |
| 5 | INPUT 1 | 13 | Signal ground |
| 6 | INPUT 2 | 14 | Signal ground |
| 7 | INPUT 3 | 15 | Signal ground |
| 8 | INPUT 4 | | |

**Next injection input:**
This input will start the next injection sequence when the Triathlon is started in remote control. When the injection sequence is finished the Triathlon will wait for the next input.

From the Ready Menu a NEXT INJECTION INPUT will start the last programmed series. In this case the Triathlon will not wait for the NEXT INJECTION INPUT before continuing with the next injection. The Triathlon will execute the complete RUN as if it was started with the **Start/Stop** key.

**Next vial input:**
With this input the Triathlon will perform the next injection from the next vial, even if not all injections from that vial in the programmed injection method have been executed.

**Freeze input:**
The Triathlon will freeze the analysis time for the time this input is active. If the FREEZE INPUT is activated while the analysis time is not running, the Triathlon will perform all programmed pre-injection sample handling (mix method and loading part of the injection method). But the Triathlon will wait with injecting the sample until the FREEZE INPUT is no longer active.

**Stop I/O:**
With this input the run of the Triathlon is immediately aborted. The Ready Menu appears in the display. In case the Triathlon is in remote control, the run of the Triathlon is immediately aborted but the Triathlon remains in remote control and cannot be restarted with a NEXT INJECTION INPUT.

**INPUT 1-4:**
Programmable input, can be used in the user program.

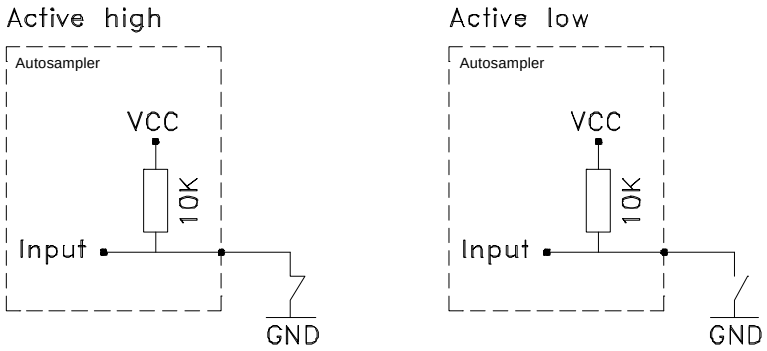
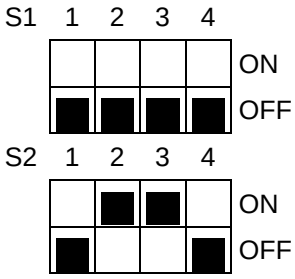



*Figure 6.3 TTL input*

## *6.4 MultiLink connector*

The MultiLink connector is used to make the Triathlon communicate with the SparkLink software.

Set the dipswitch S1 and S2 as follows to let the communication work probably:



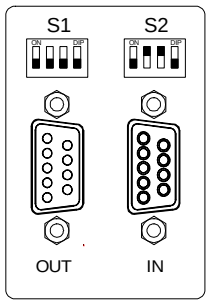




Figure 6.4: MultiLink board

| Port | | Description |
|---|---|---|
| S1: | OUT | to next device |
| S2: | IN | to PC or previous device |

Connections for 9 pin connector:

| | | |
|---|---|---|
| pin 2 | TD | Transmitted data to the computer. |
| pin 3 | RD | Received data from the computer. |
| pin 5 | SG | Signal ground (Also indicated as GND in some devices) |

# Appendix A. Installation

The Triathlon shipping container is supplied with a packing list. Please check that all items mentioned in the list are included in the package before you start the installation procedure for the Triathlon.

Do not install the Triathlon in areas subject to shock, dust, or in direct sunlight. Do not place it near a source of heat, as this will disturb tray cooling (if this option is installed).

The Triathlon must only be connected to power sources and apparatus with protective grounding.

Execute the following steps to install the Triathlon:

1. Lift the Triathlon from the shipping container. Make sure you keep the apparatus upright; lift it by placing your hands under the Triathlon.
2. Place the Triathlon in its operating location, preferably on the left hand side of the HPLC system. Make sure the ventilation holes are not obstructed. Allow the instrument to acclimatize for 1 hour.
3. Install tray (figure 1.1, number 6) in the Triathlon.
4. Check whether local voltage matches voltage indicated on back panel of the Triathlon.
5. Connect the power cord to the Triathlon (figure 1.2, number 5).
6. Switch the Triathlon on by using the switch at the back of the apparatus (figure 1.2, number 4).

The Triathlon now starts up. The display indicates that a self-test and initialization procedure are executed. After completion of this procedure the following appears on the display:

```
10:26 TUESDAY DECEMBER 16-97 [MENU]
              READY (x.xx)

<ADVANCE>  <WASH>  <SYR END>  <UTILS>
```

## *Factory installed items*

The Triathlon is factory-installed with:

- fuses in power switch:
  115 V (AC) ± 10%: two 5 AT fuses (slow, ¼" x 1¼", UL/CSA) or
  230 V (AC) ± 10%: two 2.5 AT fuses (slow, 5 x 20 mm, IEC127).
- 100 µL loop
- 250 µL syringe.
- 500 µL buffer tubing
- stainless steel 15 µL sample needle

The fuses used are UL-listed and CSA-certified.

| **Tubing** | **Material and dimensions** |
|---|---|
| Standard sample needle and tubing | SS tubing; 135 mm x 0.65 mm O.D. x 0.25 mm I.D.<br>Tefzel tubing; 140 mm x $^{1}/_{16}$" O.D. x 0.25 mm I.D.<br>(total volume 15 µL) |
| Buffer tubing from high pressure valve to syringe valve | PTFE tubing; 640 mm x $^{1}/_{16}$" O.D. x 1.0 mm I.D.<br>(volume 500 µL) |
| Tubing syringe valve to wash solvent bottle | PTFE tubing; 300 mm x $^{1}/_{16}$" O.D. x 1.0 mm I.D. |
| Tubing syringe valve to waste | PTFE tubing; 400 mm x $^{1}/_{8}$" O.D. x 1.6 mm I.D. |

For installation of HPLC connections, waste tubing, wash solvent, syringe, sample needle, trays, etc. refer to Chapter 2 of this manual.

# *Appendix B. Specifications*

## *General*

| | |
|---|---|
| Sound pressure level | LeAq < 70 dB |
| Working temperature | 10 - 35°C |
| Storage temperature | -25 / +60°C |
| Humidity | 20 - 80% RH |
| Sample viscosity | 0.1 - 5 cP |
| Safety | according to IEC 1010 |
| Sample capacity | 96 vials of 1.5 mL (STD), 12 vials per segment<br>72 vials of 4.0 mL (LSV), 9 vials per segment<br>160 microvials of 0.5 mL, 20 vials per segment<br>32 vials of 10.0 mL, 4 vials per segment |
| Tray segment types | 4 types of tray segments (see figure 2.4):<br>Type A: Standard tray segment<br>Type B: LSV tray segment<br>Type C: Super LSV tray segment<br>Type D: Micro vial tray segment |
| Vial diameter | 12 mm (Standard tray), 15 mm(LSV tray), 22 mm (Super LSV) and 7 mm (Micro tray) |
| Dimensions Vial | Maximum height: 47 mm (including cap); minimum height: 32 mm (including cap). |
| Loop volume | 5-1000 µL |
| Puncturing accuracy needle | ± 0.6 mm |
| Pre-puncturing septa/caps | With air needle, dual needle action |
| Vial & height detection | Missing vial & height detection by vial sensor |
| Switching time injection valve | Electrically < 100 msec |
| Headspace pressure | Built-in compressor |
| Wash solvent | 250 mL internal wash solvent bottle |
| Dispenser syringe | 100 µL , 250 µL (standard), 500 µL, 1000 µL, or 10 mL |
| Wetted parts | SS316, PTFE, TEFZEL, VESPEL, Glass, Teflon<br>(Optional PEEK, Fused silica) |

## *Analytical performance*

| **Capped and sealed vials:** | **Reproducibility** |
|---|---|
| • flushed loop injections | RSD ≤ 0.3 % |
| • partial loopfill injections | RSD ≤ 0.5 %, injection volumes > 5 µL, with headspace pressure on the vial and 30 µL pre-flush with air segment. |
| • µL pick-up injections | RSD ≤ 1.0 %, injection volumes > 5 µL, with headspace pressure on the vial. |
| Memory effect | < 0.01 % with programmable needle wash |

## *Programming*

| | |
|---|---|
| Injection methods | Flushed loop injections<br>Partial loopfill injections<br>µL pick-up injections |
| Injection volume | 1 µL – 1 mL, full loop, depending on system settings<br>1 - 500 µL, with 1 µL increments for partial loopfill<br>1 µL - max. volume, with 1 µL increments for µL pick-up<br>max. volume = ((loop volume - 3 x needle volume)/2) |
| Injections per vial | Max. 9 (volumes are programmable for each injection) |
| Analysis time | Max. 9 hrs. 59 min. 59 sec. |
| Needle wash | Programmable (between injections, vials or series) |
| Priority sample | Freely programmable |
| Series | Freely programmable, 24 series max. |

## *Physical*

| | |
|---|---|
| Dimensions (W x D x H) | 280 mm x 540 mm x 440 mm |
| Weight | 22 kg<br>30 kg if cooling option is installed |

## *Electrical*

| | |
|---|---|
| Power requirements | 115 VAC; ± 10 %; 50 Hz/60 Hz; 250 VA<br>230 VAC; ± 10 %; 50 Hz/60 Hz; 250 VA |
| Fuses | For 115 VAC; two 5.0 AT-fuses<br>(¼" x ¼", UL/CSA)<br>For 230 VAC; two 2.5 AT-fuses<br>(5 x 20 mm, IEC 127)<br>All fuses UL-listed and CSA-certified |
| Internal fuse | One 6.3 AT fuse<br>(5 x 20 mm, IEC 127) |

## *Communication*

| | |
|---|---|
| OUTPUTS | Inject marker<br>Vial marker<br>Labeled vial marker<br>Stop I/O<br>BCD output<br>4 Auxiliary outputs<br>2 Programmable outputs<br>Alarm output<br>4 Bit timebase |

| | |
|---|---|
| INPUTS | Next injection input<br>Next vial input<br>Freeze input<br>Stop I/O<br>4 Programmable inputs |
| SERIAL COMMUNICATION PORT | RS232C |
| PC-CONTROL | Microsoft Windows ‘95 / Windows NT |
| SPARKLINK PROTOCOL | Yes |

## *Options*

| | |
|---|---|
| Sample cooling | Built-in Peltier cooling processing unit<br>Programmable Range : 4° C - 40° C<br>Accuracy: " 2° C<br>Cooling capacity: Ambient -20° C (measured on cooling tray)<br>Note: Ring temperature at relative humidity of 80% and ambient temperature of 31° C, decreasing to 50% at 40° C |
| Integrated stream switching (ISS) | One or two optional high pressure 6-port valves |
| Solvent selection valve (SVV) | 6-way integrated solvent selection valve |
| Biocompatible option | Rheodyne 9000 series biocompatible valve, with PEEK sample needle and PEEK loop |
| Biocompatible ISS valve | One or two optional Rheodyne 9000 series biocompatible high pressure 6-port valves |
| Micro option | Triathlon for small injection volumes, down to 0.1 µl (equipped with VALCO valve, fused silica needle and special software) |
| Optional trays | LSV-tray: 9 vials of 4.0 mL per segment<br>Micro-tray: 20 micro vials per segment<br>Super LSV-tray: 4 vials of 10 mL per segment |
| Column oven | Mistral column oven combined with the Triathlon. The injection valve and optional high pressure valve fit into the column oven |

# *Appendix C. Error Codes*

The Triathlon will display an **error message** if the user tries to enter invalid data. Information on the allowed range will be displayed. If something goes wrong in the physical operations of the Triathlon, an **error code** will be displayed. Press the **Start/Stop** key twice to lift the message and try to repair the failure condition with the help of the explanation of the code concerned.

## *Injection valve and ISS unit*

| | |
|---|---|
| ERROR 11 | Injection valve is not in a valid position. |
| ERROR 12 | The injection valve did not switch within 1.5 seconds. |
| ERROR 13 | The switching time of the injection valve exceeds 500 msec. |
| ERROR 14 | ISS A valve is not in a valid position. |
| ERROR 15 | The ISS A valve did not switch within 1.5 seconds. |
| ERROR 17 | ISS valve B is not in a valid position. |
| ERROR 18 | ISS valve B did not switch within 1,5 seconds. |

## *Syringe dispenser unit*

| | |
|---|---|
| ERROR 21 | The syringe valve did not switch. |
| ERROR 22 | The syringe did not reach home position in time. |
| ERROR 23 | The syringe spindle did not make the correct number of rotations. |
| ERROR 24 | The spindle does not rotate. |
| ERROR 25 | The syringe valve did not find a valid position. |

## *Injection needle unit*

| | |
|---|---|
| ERROR 30 | The sample needle arm did not reach or leave home position (vertical). |
| ERROR 31 | The sample needle arm is in an invalid horizontal position while moving down. |
| ERROR 32 | The sample needle arm did not reach or leave destination within a certain time (horizontal). |
| ERROR 33 | The sample needle arm needed too many or too few steps to reach destination. |
| ERROR 34 | Sample needle arm not in vertical home position while moving horizontally. |
| ERROR 35 | The sample needle is at an invalid horizontal position. |
| ERROR 36 | The sample needle detects too many code gaps while moving to the next position. |
| ERROR 37 | The samlpe tray is not at a valid position while moving down the needles. Stop sensor is not in tray gap. |
| ERROR 39 | Vial sensor sticks. |
| ERROR 40 | The sample needle spindle does not rotate correctly. |
| ERROR 41 | The sample needle did not reach or leave home position. |
| ERROR 42 | The sample needle is not at home position. |

## *Tray*

| | |
|---|---|
| ERROR 51 | Incorrect tray rotation. |
| ERROR 52 | No segment found. |
| ERROR 53 | The sample needle arm is not in the home position while moving the tray. |

## *Vials*

| | |
|---|---|
| ERROR 60 | Missing vial. Only available when Skip Missing Vial is set to NO in the System Settings. |
| ERROR 61 | Missing segment.(Only available when Skip Missing Vial is set to NO in the System Settings and during the execution of a Mix Method). |
| ERROR 62 | Missing transport vial. |
| ERROR 63 | Missing transport segment. (Only available when Skip Missing Vial is set to NO in the System Settings and during the execution of a Mix Method). |
| ERROR 64 | Missing vial for reagent A. |
| ERROR 65 | Missing vial for reagent B. |
| ERROR 66 | Missing vial for reagent C. |
| ERROR 67 | Missing vial for reagent D. |
| ERROR 68 | Missing destination vial. |
| ERROR 69 | Not enough transport liquid available due to missing transport vials or segments. |

## *Electronics*

| | |
|---|---|
| ERROR 71 | Flexprint of the sample needle is not connected. |
| ERROR 72 | Invalid configuration of the Triathlon, PCB missing. |
| ERROR 73 | Current limit of the external I/O exceeded. |
| ERROR 75 | Error occurred during initialization, the Triathlon cannot start. |

# *Appendix D. Options and accessories*

The following configuration options are available for the Triathlon:

| number | description |
|---|---|
| 0900.000 | Triathlon Autosampler |
| 0900.830 | Integrated Stream Switching option, two six-way valves |
| 0900.831 | ½ Integrated Stream Switch option, one six-way valve |
| 0900.832 | Triathlon SSV: Solvent Selector Valve (SSV), a six-way solvent selector valve |
| 0900.836 | Triathlon Integrated Stream Switch, two six-way biocompatible Rheodyne 9000 series valves |
| 0900.840 | Tray cooling, Peltier type cooling elements |
| 0900.872 | Biocompatible option, Rheodyne 9000 series injection valve, Peek needle and Peek loop |
| 0900.978 | Micro option Triathlon |
| 0880.914 | Mistral, HPLC column thermostat to be used in combination with the Triathlon autosampler |
| 0880.915 | Mistral, Peltier cooled HPLC column thermostat to be used in combination with the Triathlon autosampler |

Trays and tray segments:

| | |
|---|---|
| 0900.313 | Tray set (for cool option); 8 segments, 1.8 mL vial, 12 positions, 12 mm O.D. |
| 0900.315 | Tray set (standard); 8 segments, 1.8 mL vial, 12 positions, 12 mm O.D. |
| 0900.314 | Tray segment, 0.5 mL vial, 20 positions, 7 mm O.D. |
| 0900.316 | Tray segment, 4 mL vial, 9 positions, 15 mm O.D. |
| 0900.317 | Tray segment, 10 mL vial, 4 positions, 22 mm O.D. |
| 0900.351 | Tray segment; standard tray, number 1 |
| 0900.352 | Tray segment; standard tray, number 2 |
| 0900.353 | Tray segment; standard tray, number 3 |
| 0900.354 | Tray segment; standard tray, number 4 |
| 0900.355 | Tray segment; standard tray, number 5 |
| 0900.356 | Tray segment; standard tray, number 6 |
| 0900.357 | Tray segment; standard tray, number 7 |
| 0900.358 | Tray segment; standard tray, number 8 |
| 0900.361 | Tray segment; tray with cooling option, number 1 |
| 0900.362 | Tray segment; tray with cooling option, number 2 |
| 0900.363 | Tray segment; tray with cooling option, number 3 |
| 0900.364 | Tray segment; tray with cooling option, number 4 |
| 0900.365 | Tray segment; tray with cooling option, number 5 |
| 0900.366 | Tray segment; tray with cooling option, number 6 |
| 0900.367 | Tray segment; tray with cooling option, number 7 |
| 0900.368 | Tray segment; tray with cooling option, number 8 |

**number description**

**The following accessories are available for the Triathlon:**

| number | description |
|---|---|
| 0043.344 | Tray cooling cover |
| 0043.364 | Needle wash insert vial |
| 0043.473 | Needle body |
| 0060.123 | User manual |
| 0700.002 | Long Bushing Rheodyne (pck/10) |
| 0700.003 | Ferrule Rheodyne (pck/10) |
| 0700.008 | Rheflex ferrule (pck/10) |
| 0700.009 | Rheflex nut (pck/10) |
| 0700.010 | Flanged tube fitting 1/8 (pck/5) |
| 0700.011 | Flanged tube fitting 1/16 (pck/5) |
| 0700.012 | Plunger replacement tip 100 µL (pck/10) |
| 0700.018 | Plunger replacement tip 500 µL (pck/10) |
| 0700.019 | Plunger replacement tip 1 mL (pck/10) |
| | |
| 0900.300 | Biocompatible injection valve assembly |
| 0900.301 | Injection valve assembly |
| 0900.303 | Sample needle including tubing ferrules and nuts |
| 0900.304 | Serum sample needle including tubing ferrules and nuts |
| 0900.311 | Peek sample needle including tubing ferrules and nuts |
| 0900.321 | Sample needle extra long including tubing ferrules and nuts |
| 0900.322 | Serum sample needle extra long including tubing ferrules and nuts |
| 0900.323 | Peek sample needle extra long including tubing ferrules and nuts |
| 0900.325 | Fused silica needle including tubing ferrules and nuts |
| 0900.326 | Fused silica needle extra long including tubing ferrules and nuts |
| 0900.700 | Software Triathlon |
| 0900.710 | Buffer tubing 500 µL |
| 0900.711 | Buffer tubing 2000 µL |
| 0900.712 | Syringe waste tubing |
| 0900.713 | Syringe wash tubing |
| 0900.714 | Tubing Solvent Selector valve, Syringe valve |
| 0900.715 | Buffer tubing 15 mL |
| 0900.718 | Air/prepuncturing needle |
| 0900.721 | Tefzel buffer tubing 2000 µL |
| 0900.811 | Inject marker cable |
| 0900.812 | Next injection input cable |
| 2140.160 | Luerlock connection syringe valve |
| 3796.004 | Rheodyne 7010 rotor seal |
| 3796.005 | Rheodyne 7010 stator |
| 3796.035 | Rheodyne loop 5 µL |
| 3796.009 | Rheodyne loop 10 µL |
| 3796.010 | Rheodyne loop 20 µL |
| 3796.019 | Rheodyne loop 50 µL |

| number | description |
|---|---|
| 3796.012 | Rheodyne loop 100 µL |
| 3796.013 | Rheodyne loop 200 µL |
| 3796.014 | Rheodyne loop 500 µL |
| 3796.015 | Rheodyne loop 1000 µL |
| 3796.016 | Rheodyne loop 100 µL Peek |
| 3796.076 | Rheodyne loop 20 µL Peek |
| 3796.032 | Rheodyne Rheflex ferrule |
| 3796.033 | Rheodyne Rheflex nut |
| 3796.040 | Rheodyne 9010 Rotor seal |
| 3796.041 | Rheodyne 9010 Stator |
| 3796.042 | Rheodyne Stator face assembly |
| 3796.050 | Rheodyne 7010 Tefzel rotor seal |
| 3796.062 | Flanged tubing fitting 1/8 " |
| 3796.065 | Flanged tubing fitting 1/16" |
| 4340.025 | Wash solvent bottle 250 mL |
| 4400.000 | Syringe 1000 µL |
| 4400.030 | Syringe100 µL |
| 4400.250 | Syringe 250 µL |
| 4400.500 | Syringe 500 µL |
| 4400.251 | Syringe 25 µL (for micro option Triathlon) |
| 4400.001 | Syringe 10 mL |
| 4400.601 | Plunger replacement tip 100 µL (pck/10) |
| 4400.602 | Plunger replacement tip 250 µL (pck/10) |
| 4400.603 | Plunger replacement tip 500 µL (pck/10) |
| 4400.604 | Plunger replacement tip 1000 µL (pck/10) |
| 4400.607 | Plunger replacement tip 25 µL (pck/10) (micro option) |
| 4400.605 | Plunger replacement o-ring 500 µL (pck/10) |
| 4400.606 | Plunger replacemnt o-ring 1000 µL (pck/10) |

# Appendix E. Calibration and performance

The Triathlon is factory tested for reproducibility, carry-over and mixing according to the following test procedure.

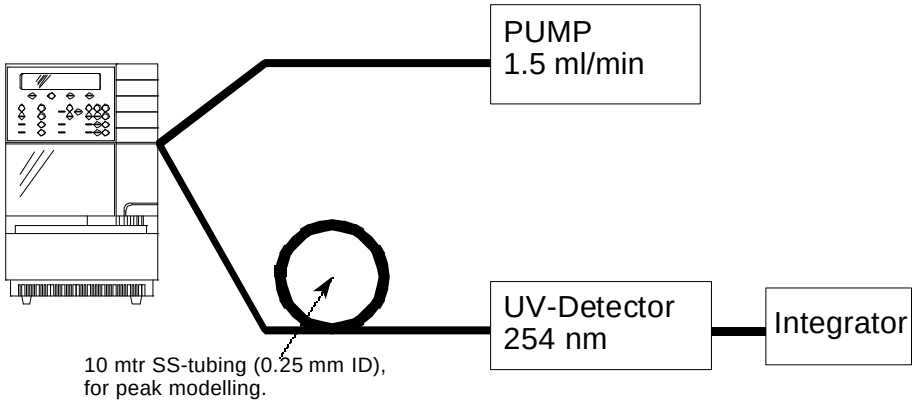
## E.1 Analytical performance test

### E.1.1 Analytical system

The Triathlon is tested in an analytical system under the following conditions:

| | | |
|---|---|---|
| Pump | flow: | 1.5 mL/min |
| | eluent: | distilled water |
| Detector | wavelength: | 254 nm |
| Sample: | | Uracil in distilled water (50 ppm) |

The following configuration was used:




### E.1.2 Relative standard deviation (RSD%)

The following formulas are required for calculating the RSD:

$$\overline{Peak\ area} = \frac{\sum Peak\ area}{n}$$

$$\sigma_{n-1} = \sqrt{\frac{\sum \left(Peak\ area - \overline{Peak\ area}\right)^2}{n-1}}$$

$$RSD\% = \frac{\sigma_{n-1}}{Peak\ area} \times 100\%$$

## *E.1.3 Reproducibility test*

The default system settings were used, except for the following:

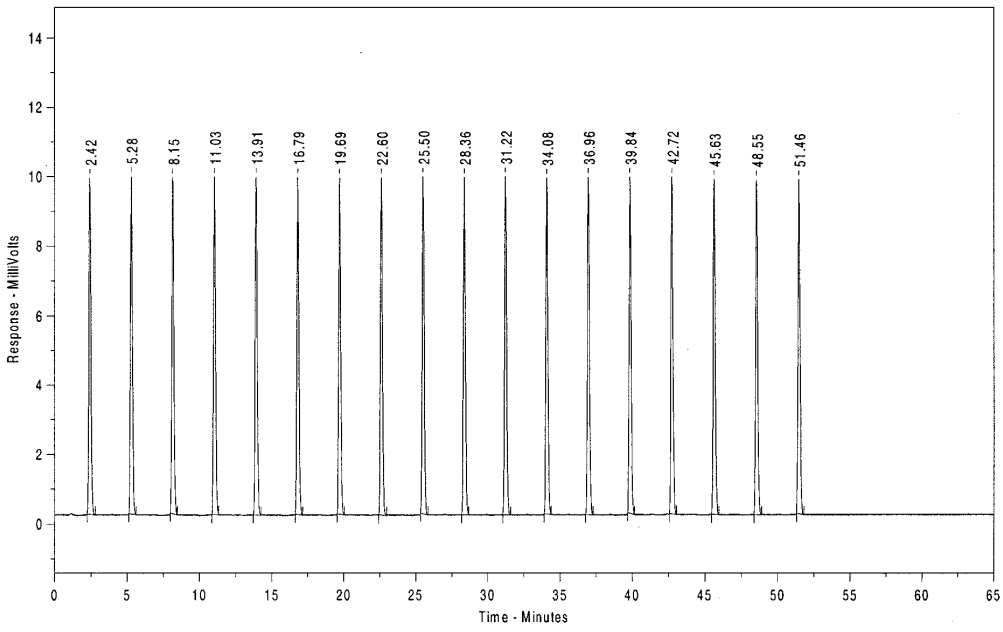
| | |
|---|---|
| <GENERAL> | Air segment: NO |
| <TRAYS> | Location first transport vial: A7 |
| | Location last transport vial: A7 |

Sx vials are filled with sample on positions A1 - A6.
The transport solvent vial is filled with eluent and placed on position A7.

The wash solvent bottle is filled with 80% $H_2O$ / 20% Iso-propanol.

| **Injection method** | | **Wash method** | | **Series** | |
|---|---|---|---|---|---|
| Number | 1 | Number | 1 | Number | 1 |
| Type | µL pick-up | Wash between injections | | First vial | A 01 |
| Analysis time | 1:00 min | Wash volume | 300 µL | Last vial | A 06 |
| Injections/vial | 3 | | | Injection method | 1 |
| Injection volume 1 | 5 µL | | | Wash method | 1 |
| Injection volume 2 | 5 µL | | | | |
| Injection volume 3 | 5 µL | | | | |

Example chromatogram of the reproducibility test:



| Peak number | Area |
|---|---|
| 1 | 206342 |
| 2 | 204773 |
| 3 | 205425 |
| 4 | 205203 |
| 5 | 205566 |
| 6 | 205568 |
| 7 | 206156 |
| 8 | 206174 |
| 9 | 205956 |
| 10 | 206165 |
| 11 | 205765 |
| 12 | 205960 |
| 13 | 206296 |
| 14 | 205617 |
| 15 | 205619 |
| 16 | 205648 |
| 17 | 206147 |
| 18 | 207070 |

From the integration results the RSD% for the Triathlon can be calculated.

For this example the results are:

RSD% = 0.25 %

The specified value is an RSD% < 1%.

## *E.1.4 Mixing and carry-over test*

The default system settings were used, except for the following:

<USAGE> Use of mix methods: ENABLED

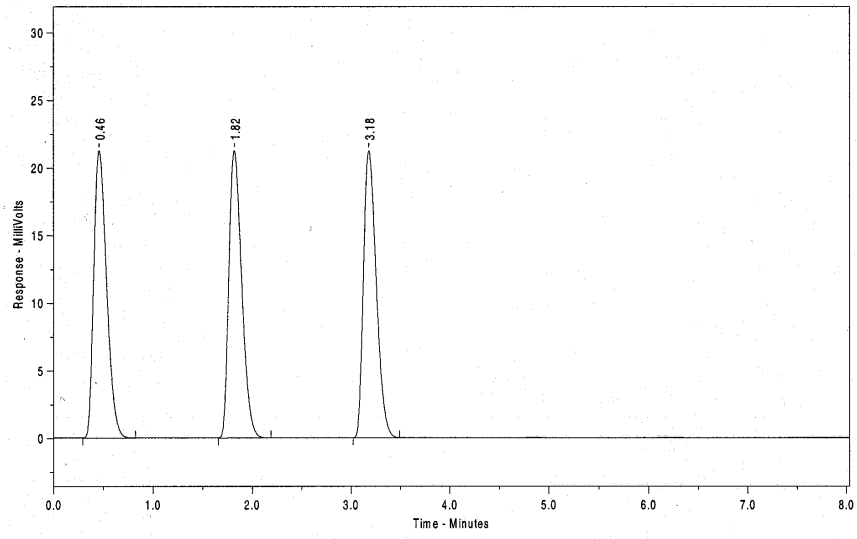
A tray with one sample vial filled with sample (50 ppm Uracil, A01), one sample vial filled with eluent (A02), and one empty (destination, A03) vial are placed in the Triathlon. A 1:10 dilution of sample from the sample vial will be made in the empty destination vial. After dilution from both vials (sample and destination) 10 µL will be injected. The Reagent-A vial is filled with eluent.
The wash solvent bottle is filled with 80% $H_2O$ / 20% Iso-propanol.

| **Injection method** | | **Series 1** | | **Series 2** | |
|---|---|---|---|---|---|
| Number | 1 | Number | 1 | Number | 2 |
| Type | partial loopfill | Injection method | 1 | Injection method | 1 |
| Analysis time | 1:00 min | Wash method | None | Wash method | none |
| Flush volume | 35 µL | Mix method | 1 | Mix method | none |
| Injections/vial | 3 | First vial | A 01 | First vial | A 01 |
| Injection volume 1 | 10 µL | Last vial | A 01 | Last vial | A 02 |
| Injection volume 2 | 10 µL | First destination vial | A 03 | | |
| Injection volume 3 | 10 µL | Reagent A vial | A 02 | | |

**Mix method 1**

| **Step** | **Action** | | | **Speed** | **Height** |
|---|---|---|---|---|---|
| 1 | Aspirate | 5 µL | Air | 2 | |
| 2 | Aspirate | 225 µL | Reagent A | 2 | 02 |
| 3 | Dispense | 180 µL | to Destination | 3 | 02 |
| 4 | Dispense | 50 µL | to Waste | 5 | |
| 5 | Repeat last 4 steps 1 time | | | | |
| 6 | Aspirate | 5 µL | Air | 2 | |
| 7 | Aspirate | 60 µL | Sample | 2 | 02 |
| 8 | Dispense | 40 µL | to Destination | 3 | 02 |
| 9 | Dispense | 50 µL | to Waste | 5 | |
| 10 | Aspirate | 50 µL | Air | 2 | |
| 11 | Aspirate | 200 µL | from Destination | 3 | 0 |
| 12 | Dispense | 200 µL | to Destination | 9 | 20 |
| 13 | Repeat last 2 steps 3 times | | | | |
| 14 | End of mix method | | | | |

Example chromatogram of the mixing and carry-over test:



| Peak number | Area |
|---|---|
| 1 | 38802 |
| 2 | 38968 |
| 3 | 39088 |
| 4 | 395258 |
| 5 | 394090 |
| 6 | 394471 |
| 7 | - |
| 8 | - |
| 9 | - |

From the integration results the RSD% for the two vials and the carry-over for the Triathlon can be calculated.

For this example the results are:
RSD% (vial A01, sample) = 0.15 %
RSD% (vial A03, 1:10 dilution) = 0.37 %

1:10 dilution = 1:10.13: variation -1.29%
The specified variation is ± 5%

Carry-over (vial A02): < 0.2%
The real value cannot been calculated, the specified value is a Carry-over <0.2%.

## *E.2 Syringe calibration*

To calibrate the syringe dispense a volume of 200 µL water from a sample vial to an empty destination vial with the following mix method.

**Mix method 1**

| Step | Action | | | Speed | Height |
|---|---|---|---|---|---|
| 1 | Aspirate | 250 µL | Sample | 2 | 02 |
| 2 | Dispense | 200 µL | to Destination | 3 | 02 |
| 3 | End of mix method | | | | |

**Series 1**

| | |
|---|---|
| Injection method | None |
| Wash method | None |
| Mix method | 1 |
| First sample vial | A 01 |
| Last sample vial | A 01 |
| First destination vial | A 02 |

- Weigh the destination vial before and after the Run.
- The difference is the dispensed volume
- The specified variation is ± 2%

## *E.3 Tray cooling calibration*

1. Place a thermocouple on the bottom of the tray, make sure the contact is good.
2. Switch on the tray cooling and program a setpoint of 10°C
3. Wait minimal 15 minutes for equilibration of the Triathlon
4. Read out the temperature of the thermocouple.

The value must be in a range of ± 2°C of the programmed setpoint.

## *E.4 Loop calibration*

1. Disconnect the loop from the injection valve.
2. Remove all liquids from the loop with air.
3. Weigh the empty loop on an analytical balance.
4. Fill the loop with minimal 2 times its volume of water.
5. Weight the filled loop again.
6. The difference in weight is the capacity of the loop.
7. The weight divided by the specific weight of water (1 g/mL) gives the calibrated volume of the loop.

Allowed variation: ± 10% (According to Rheodyne).

# Appendix F. Rheodyne injection valve

The Triathlon is standard equipped with a Rheodyne 7739 valve; the biocompatible Triathlon is equipped with a Rheodyne 9740 valve. The text below is a copy of the Rheodyne Operating instructions.

## F.1 Operating Instructions for Rheodyne Model 7739 Valve

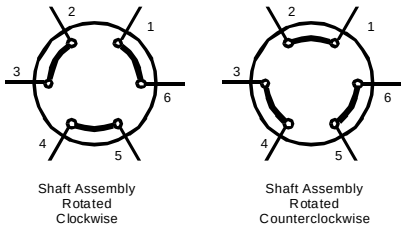



*Figure F.1 Flow diagram of Model 7739 as viewed from stator.*

Model 7739 is a two-position, six-port stainless steel valve. Figure F.1 shows the flow diagram of the valve. The six small circles represent the ports in the valve stator. The slots are the connecting passages in the rotor seal. Rotation of the valve 60° switches the valve from one position to another.

**Specifications:**
Maximum temperature is 100°C.
The valve is set to hold 345 bar (5000 psi).
The wetted surfaces are stainless steel and an inert polymer.

**Maintenance**
With normal use the valve will give many tens of thousands of cycles without trouble. The main cause of early failure, which is seen as a leak in the valve, is abrasive particles in the sample and/or mobile phase which can scratch the rotor seal. Following is the procedure for changing the rotor seal and isolation seal.
Refer to figure F.2 and proceed:

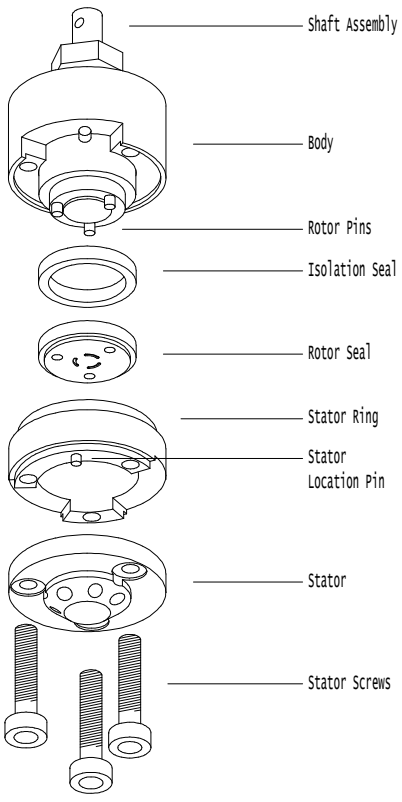



*Figure F.2 Exploded view of model 7739*

1. Remove the three stator screws with the 9/16” hex key.
2. Remove the stator and stator ring from the valve body.
3. Pull the rotor seal off the pins.
4. Remove the isolation seal.
5. Mount the new isolation seal with the spring side facing away from the rotor seal.
6. Mount the new rotor seal. The three pins on the shaft assembly fit into the mating holes in the rotor seal only one way. Mount the seal with the grooves facing the stator.
7. Replace the stator ring so that the body locating pin in the stator ring enters the mating hole in the body.
8. Mount the stator on the valve so the stator locating pin in the stator ring enters the mating hole in the stator.
9. Replace the three stator screws. Tighten each an equal amount until the screws are tight.

## *F.2 Operating Instructions for Rheodyne Model 9740 Valve*

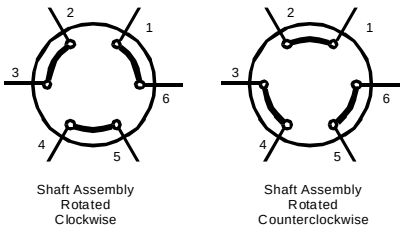



*Figure F.3 Flow diagram of model 9740 as viewed from stator.*

Model 9740 is a two-position, six-port PEEK valve. Figure F.3 shows the flow diagram of the valve. The six small circles represent the ports in the valve stator. The slots are the connecting passages in the rotor seal. Rotation of the valve 60°switches the valve from one position to another.

**Specifications**

Maximum temperature is 50°C.

The valve is set to hold 345 bar (5000 psi).

The wetted surfaces are PEEK alumina ceramic, and an inert polymer.

Use only plastic ferrules in the stator ports. Metal ferrules can cause irreparable damage to the plastic stator.

**Maintenance**

With normal use the valve will give many tens of thousands of cycles without trouble. The main cause of early failure, which is seen as a leak in the valve, is abrasive particles in the sample and/or mobile phase which can scratch the rotor seal.

Following is the procedure for changing the rotor seal, stator face assembly, and isolation seal. Refer to figure F.4 and proceed:

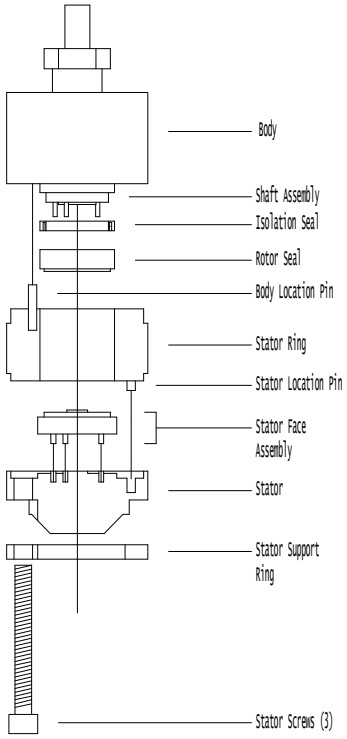
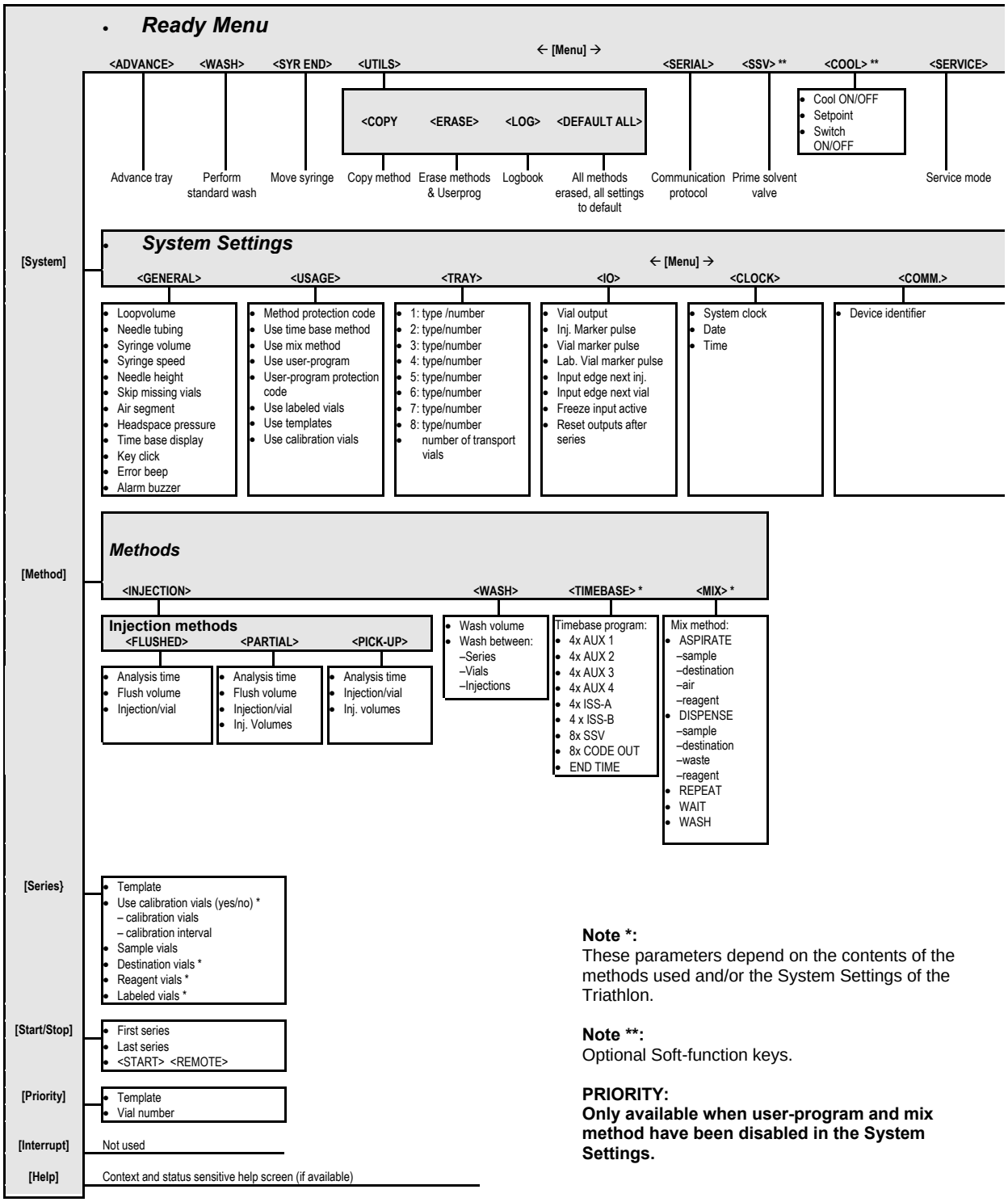



*Figure F.4 Exploded view of Model 9740*

1. Remove the three stator screws with a 9/64 inch hex key.
2. Remove the stator, stator face assembly and stator ring from the valve body.
3. Pull the rotor seal off the pins.
4. Remove the isolation seal.
5. Mount the new isolation seal with the spring side facing away from the rotor seal.
6. Mount the new rotor seal. The three pins on the shaft assembly fit into the mating holes in the rotor seal only one way. Mount the seal with the grooves facing the stator.
7. Replace the stator ring so that the body locating pin in the stator ring enters the mating hole in the body.
8. Put the new stator face assembly on the stator. The three pins on the assembly fit the mating holes in the stator only one way.
9. Mount the stator and stator face assembly on the valve so the stator locating pin in the stator ring enters the mating hole in the stator.
10. Replace the three stator screws. Tighten each an equal amount until the screws are tight.

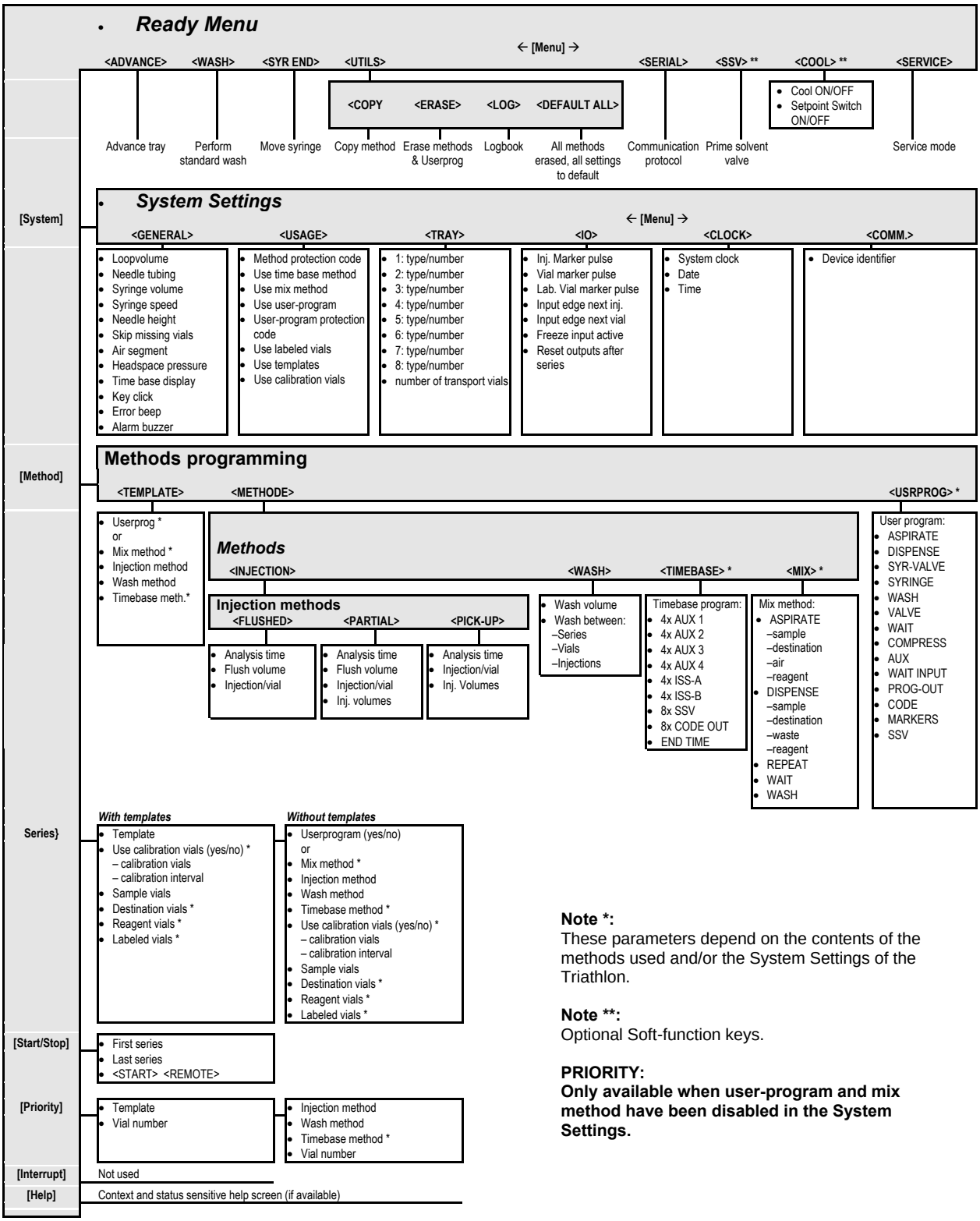
# Appendix G. Programming options




**Note *:**
These parameters depend on the contents of the methods used and/or the System Settings of the Triathlon.

**Note **:**
Optional Soft-function keys.

**PRIORITY:**
**Only available when user-program and mix method have been disabled in the System Settings.**




**Note *:**
These parameters depend on the contents of the methods used and/or the System Settings of the Triathlon.

**Note **:**
Optional Soft-function keys.

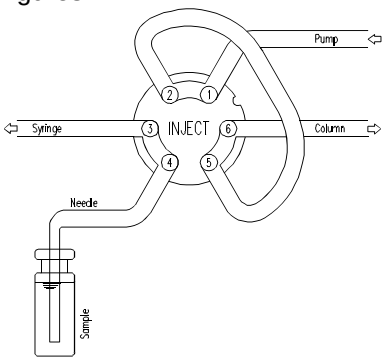
**PRIORITY:**
**Only available when user-program and mix method have been disabled in the System Settings.**

# *Appendix H. Injection principle*

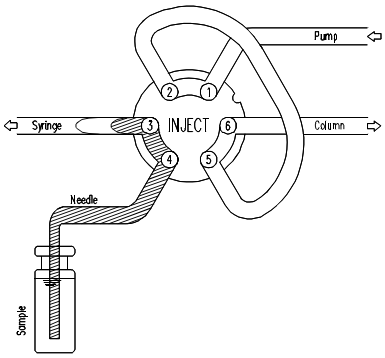
This appendix offers specialist information on the injection principles used by the Triathlon.

## *H.1 Flushed loop injections*

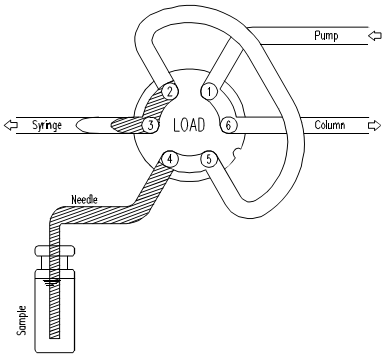
The switching sequence for a flushed loop injection is schematically shown in the following figures:



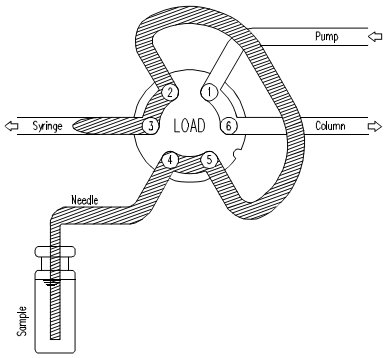

The initial situation: the injector is in the INJECT position. The sample needle has entered the vial after the air neede has prepunctured the septum. Headspace pressure is applied through the outer air needle to ensure that no air or vapor bubbles are formed during sample aspiration.



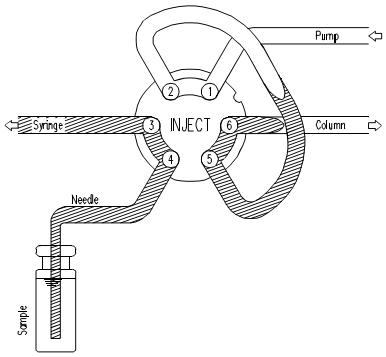

The syringe dispenser aspirates the programmed flush volume from the sample vial to fill the sample line with sample and remove wash solvents.




The injection valve switches in LOAD position, placing a "sharp" sample front at the inlet of the sample loop.




For flushed loop injections the sample loop is quantitatively filled by transporting two or more times the loop volume through the loop, depending on the volume of the loop.




The injection valve switches in INJECT position. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis time starts.

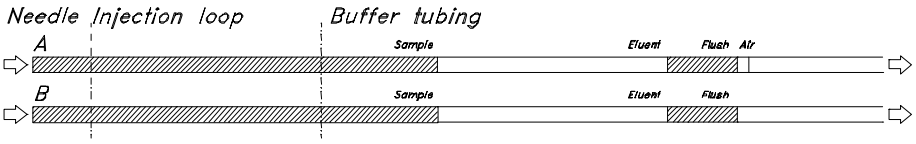
If one injection is to be done from each vial or if a wash routine has to be performed after every injection, the needle withdraws from the vial directly after the injection and, if programmed, directly performs a wash. After the analysis time a new sequence is started.

If more than one injection is done from the same vial without a wash routine, the Triathlon withdraws a flush volume after the analysis time to compensate for diffusion of mobile phase from the rotor groove into the first part of the sample line during the analysis time. The flush volume between injections is not programmable and is always 50% of the programmed flush volume. If the total amount of sample withdrawn with the next injection from the vial will exceed the total volume of the buffer tubing, the buffer tubing is emptied into the wash position before the next injection. The next fill sequence will then start with a full flush volume.

**Air segment**

An air segment can be used to reduce the amount of flush volume. This air segment is at the front of the flush volume and will not be injected and therefore will not influence the injection. Use of an air segment can be enabled in the System Menu (General Menu).

With a standard needle the flush volumes must be: minimal 30 µL for injections with air segment and 35 µL for injections without air segment. If the samples are highly viscous it may be necessary to program larger flush volumes and reduce the syringe speed for better performance.

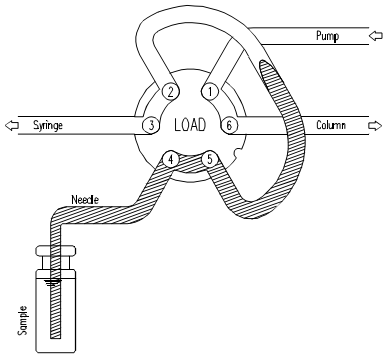



*A = with air segment; B = without air segment*

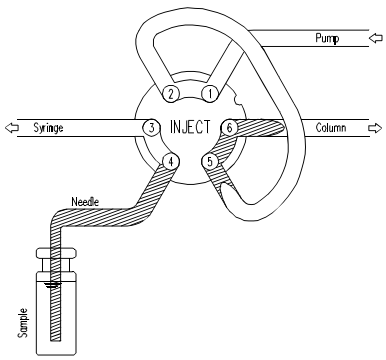
## *H.2 Partial loopfill injections*

The switching sequence for a partial loopfill injection is schematically shown in the following figures:

The first three steps are identical to those for Full loop injections (see H.1).



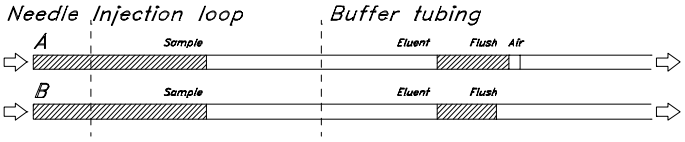

For partial loopfill injections the sample loop is filled by transporting the programmed injection volume into the sample loop.




The injection valve switches into the INJECT position. The sample loop is now part of the HPLC mobile phase flow path: the sample is transported to the column. The analysis time starts.

The next injection sequence will start with a flush of 50% of the programmed flush volume, in case an injection from the same vial and no wash routine is programmed. Otherwise it will start with a flush of the programmed flush volume. If the aspiration of sample for the next injection will exceed the total volume of the sample buffer tubing, the buffer tubing is emptied before the next injection. The next injection will start with the programmed flush, see also the full loop injections.

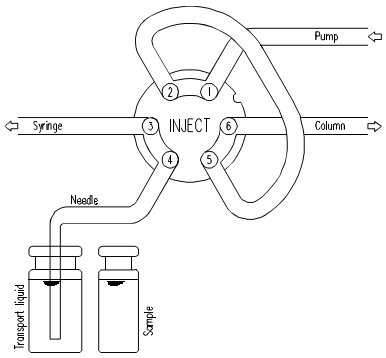
**Air segment**
An air segment can be used to reduce the amount of flush volume. This air segment is at the front of the flush volume and will not be injected. Use of an air segment can be enabled in the System Menu (General Menu).



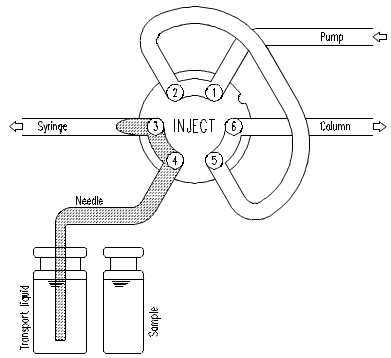

*A = with air segment; B = without air segment*

## *H.3 µL pick-up injections*

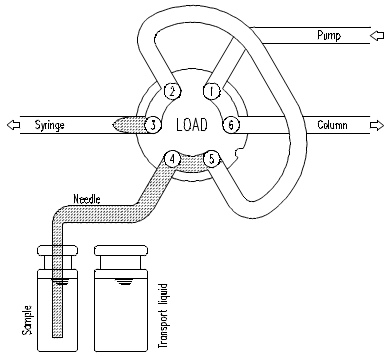
The switching sequence for a µL pick-up injection is schematically shown in the following figures:



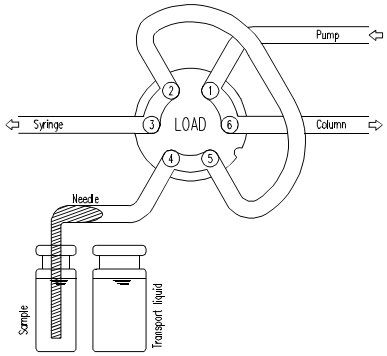

The initital situation: the injection valve is in INJECT position. The sample needle has entered the vial of transport liquid (mobile phase, to avoid disturbance of the chromatogram with an additional peak of the transport solvent) after the air needle has prepunctured the septum. The headspace pressure, applied through the outer air needle, ensures that no air or vapor bubbles are formed during wash solvent aspiration.



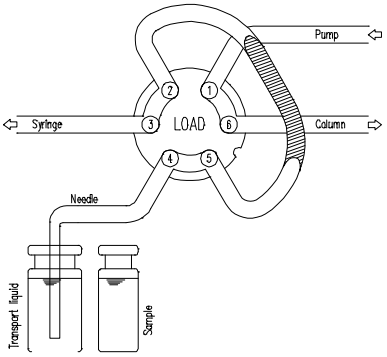

For the first injection after a wash or after emptying of the buffer tubing, the syringe dispenser aspirates transport liquid from the transport vial to fill the sample line with transport liquid and remove wash solvent.



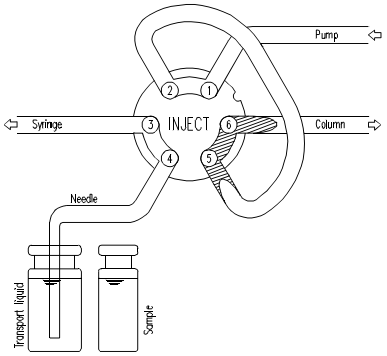

The needle moves from the transport vial to the sample vial. The injection valve is switched to the LOAD position.




The programmed injection volume is aspirated from the sample vial.




The sample needle moves back to the transport vial. The sample is quantitatively transported into the loop, with transport liquid (mobile phase) from the transport vial.



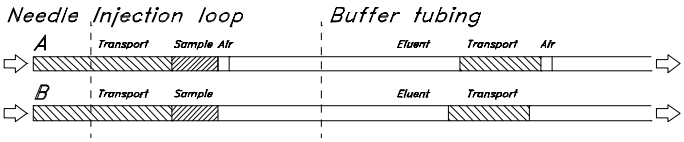

The injection valve is switched to INJECT. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis time starts to run.

The next sequence will skip the first withdrawal of transport solvent, unless a wash routine is performed or the Triathlon has emptied the buffer tubing to waste. In those cases the sequence is completely repeated.

**Air segment**

If an air segment has been programmed, it appears at the front of the first plug of transport liquid and at the front of every sample plug. Use of an air segment can be enabled in the System Menu (General Menu).

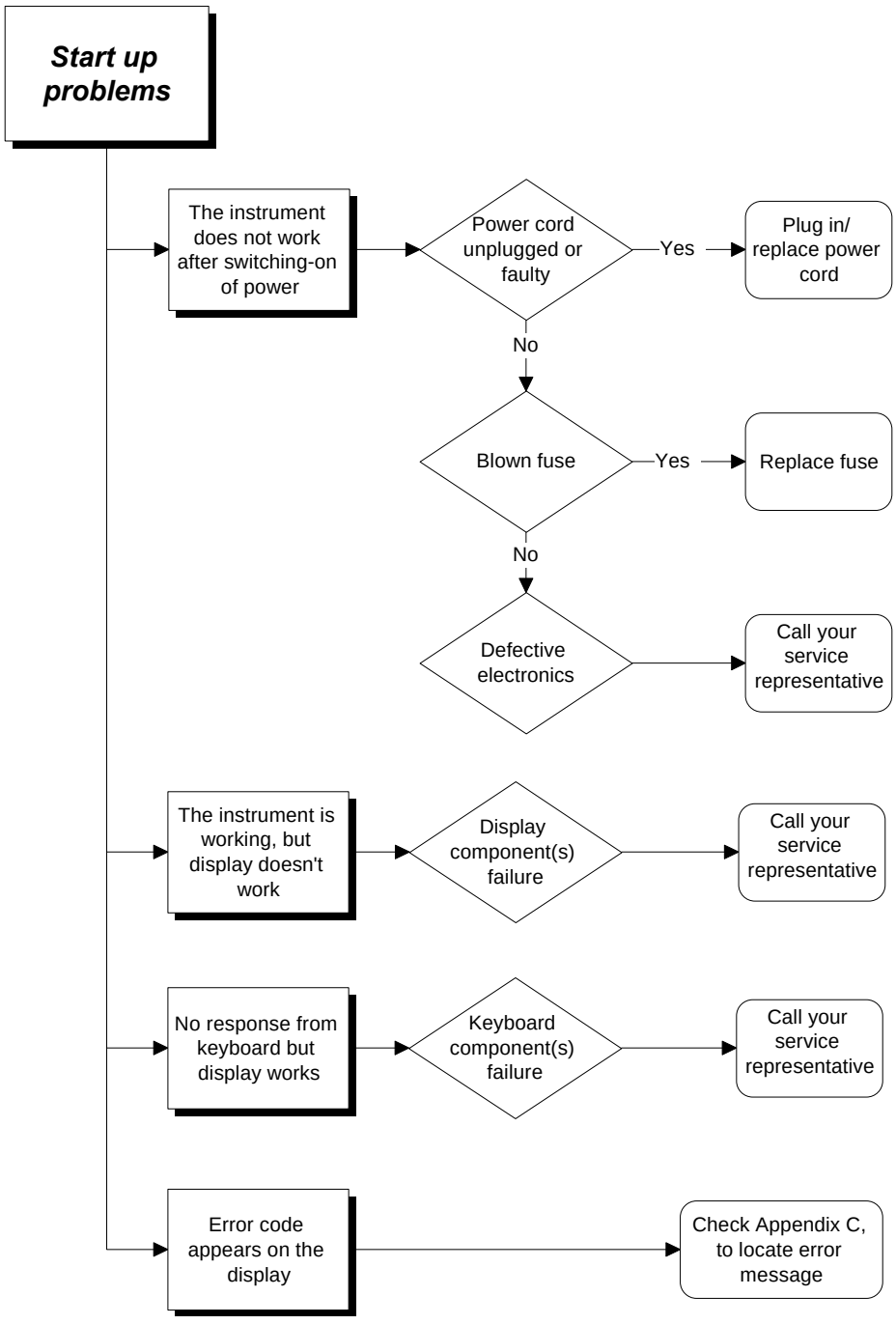
Note: The air segment at the front of the sample plug is injected into the HPLC system.




*µL pickup injections with (A) or without (B) air segment.*

In case of µL pick-up injections there will be no air pressure (headspace pressure) on the sample vial to prevent errors due to air expansion during switching from sample vial to transport vial.
You are advised to switch off use of an Air segment in the System Menu if the µL pick-up injection method is used.

# Appendix I. Trouble shooting

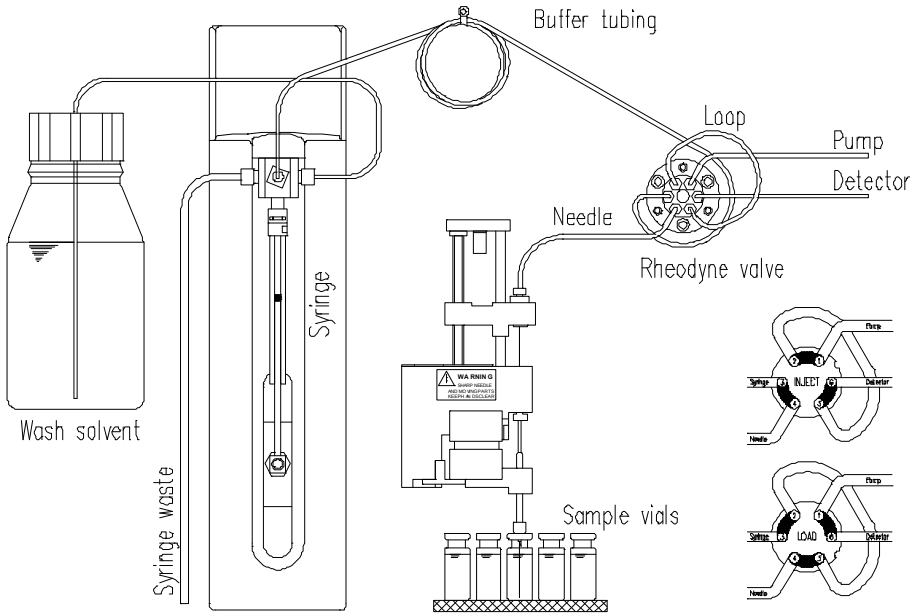


# *Analytical problems*

In case of analytical problems you will have to determine whether they are caused by the autosampler or by the rest of the system.

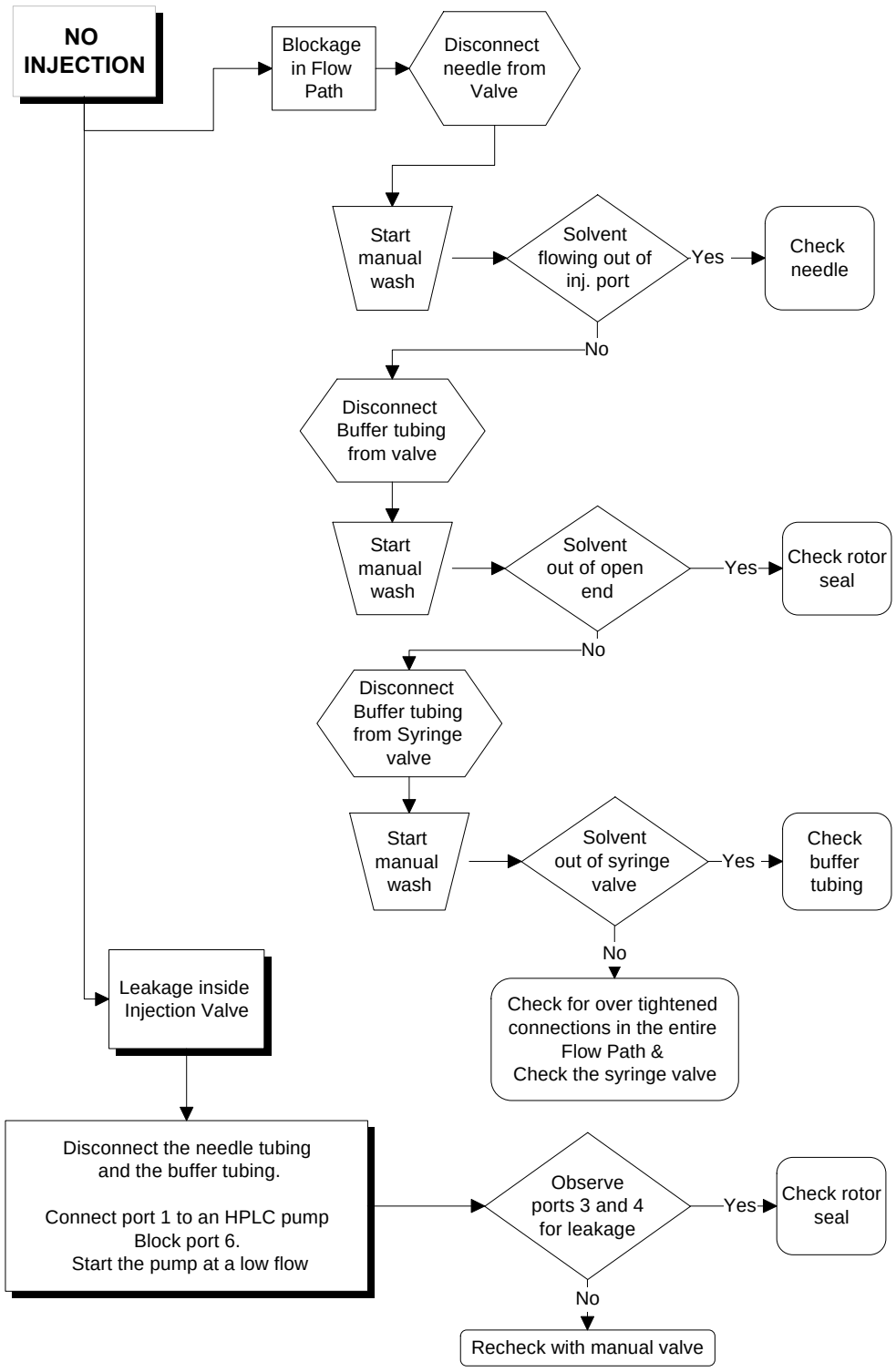
**Quick check!**
Replace the valve by a manual injection valve to discriminate between valve problems and other problems.

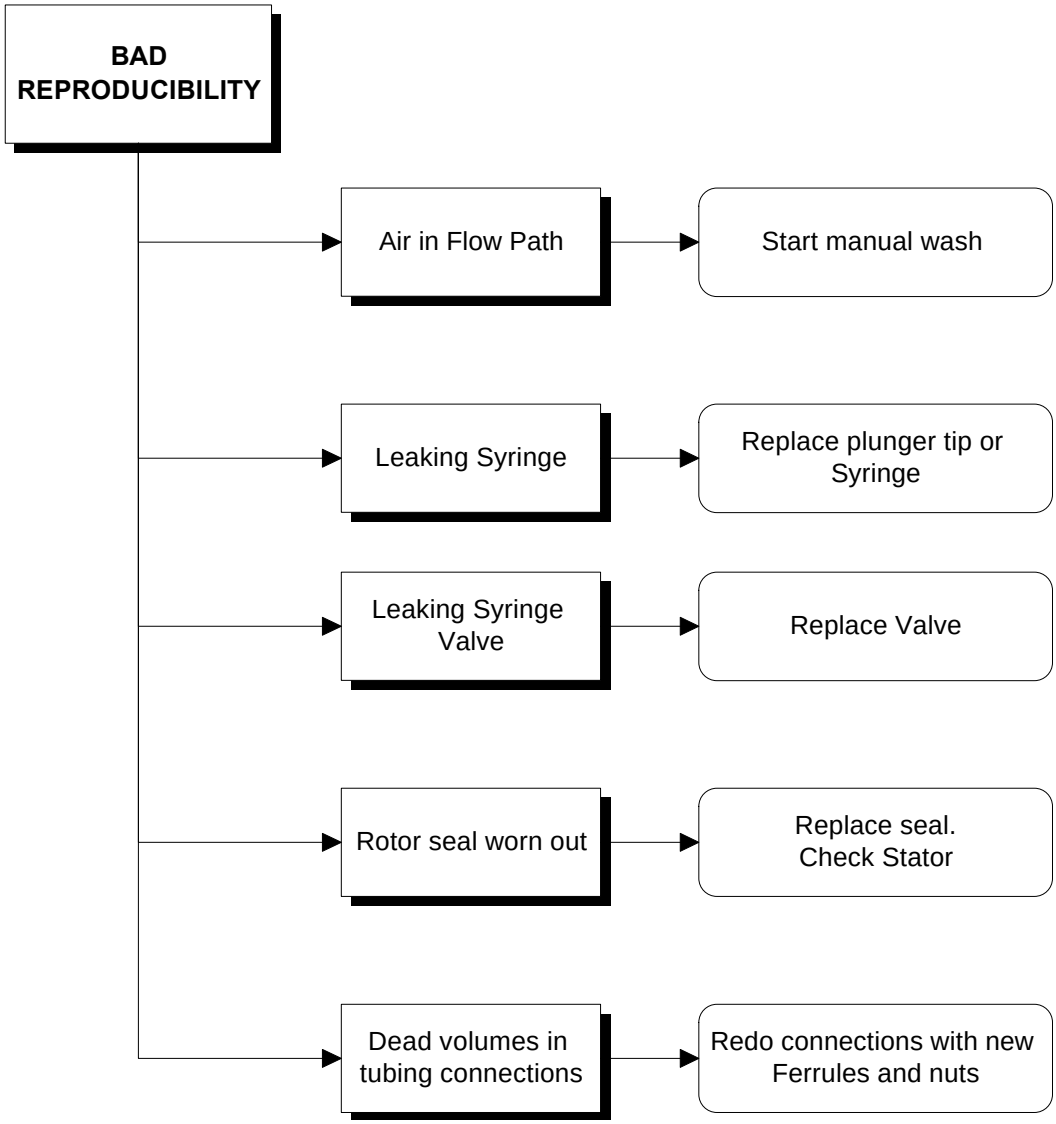
Perform a number of manual Flushed loop injections. If the results are good, the autosampler is faulty; if not, the HPLC system should be checked.




In the flowcharts on the next pages it is assumed that the Triathlon does not display any error messages.

Please keep in mind that analytical problems might be caused by external influences, like temperature and/or light-sensitive samples. For this reason it is important to make sure that the application was running without problems before and that no changes have been made in the settings (System Menu).


NO INJECTION
Blockage in Flow Path
Disconnect needle from Valve
Start manual wash
Solvent flowing out of inj. port
Yes
Check needle
No
Disconnect Buffer tubing from valve
Start manual wash
Solvent out of open end
Yes
Check rotor seal
No
Disconnect Buffer tubing from Syringe valve
Start manual wash
Solvent out of syringe valve
Yes
Check buffer tubing
No
Check for over tightened connections in the entire Flow Path & Check the syringe valve
Leakage inside Injection Valve
Disconnect the needle tubing and the buffer tubing.
Connect port 1 to an HPLC pump
Block port 6.
Start the pump at a low flow
Observe ports 3 and 4 for leakage
Yes
Check rotor seal
No
Recheck with manual valve


BAD REPRODUCIBILITY
Air in Flow Path
Start manual wash
Leaking Syringe
Replace plunger tip or Syringe
Leaking Syringe Valve
Replace Valve
Rotor seal worn out
Replace seal. Check Stator
Dead volumes in tubing connections
Redo connections with new Ferrules and nuts

# Appendix J. Logbooks

The following pages contain sample pages for logbooks that can be used with the Triathlon. Keep a record of settings (System Menu) and of programmed methods and templates (Methods Menu) for future reference. Use copies of the provided pages.

# *User information*

Name of user

Company

Department

Address

Telephone

Telefax

# *Triathlon information*

Serial number

Firmware version

Purchase date

Installed options

Local dealer

Service engineer

Address

Telephone

Telefax

***Comments:***

# *System Menu settings*

| **<GENERAL>** | | | | **<USAGE>** | |
|---|---|---|---|---|---|
| Loop volume | | | µL | Protection code: | ..... |
| | | | | Timebase methods | ☐enabled |
| Needle tubing volume | | | µL | | ☐disabled |
| | | | | | |
| Syringe volume | | | µL | Mix methods | ☐enabled |
| | | | | | ☐disabled |
| Syringe speed | .... | factor | .... | | |
| | | | | User program | ☐enabled |
| Needle height | | | mm | | ☐disabled |
| | | | | | |
| Skip missing vials | ☐yes | ☐no | | User program protection code | ..... |
| Air segment | ☐yes | ☐no | | | |
| | | | | Labeled vials | ☐enabled |
| Headspace pressure | ☐yes | ☐no | | | ☐disabled |
| | | | | | |
| Time display | HH:MM:SS | H:MM:mm | | Templates | ☐enabled |
| | | | | | ☐disabled |
| Key click | ☐on | ☐off | | | |
| Error beep | ☐on | ☐off | | Calibration vials | ☐enabled |
| Alarm buzzer | ☐on | ☐off | | | ☐disabled |

| **<TRAY>** | **<IO>** | | |
|---|---|---|---|
| ☐ 1: type /number | Inject-marker pulse length | | ....sec. |
| ☐ 2: type /number | | | |
| ☐ 3: type /number | Vial-marker pulse length | | ....sec. |
| ☐ 4: type /number | | | |
| ☐ 5: type /number | Labeled vial-marker pulse | | |
| ☐ 6: type /number | length | | ....sec. |
| ☐ 7: type /number | | | |
| ☐ 8: type /number | Input edge next injection | ☐falling | ☐rising |
| | | | |
| position first transport vial: .... | Input edge next vial | ☐falling | ☐rising |
| position last transport vial: .... | | | |
| | Freeze input active | ☐low | ☐high |
| | | | |
| | Reset outputs after last series | ☐yes | ☐no |

| **<CLOCK>** | **<COMM.>** |
|---|---|
| ☐On (yy/mm/dd and hh/mm) | Device identifier: 2.. |
| ☐Off | |

***Comments:***

# *Templates*

| Template number | Injection method | Mix method | Wash method | Timebase method | User program Y/N | Comments |
|---|---|---|---|---|---|---|
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |
| 6 | | | | | | |
| 7 | | | | | | |
| 8 | | | | | | |
| 9 | | | | | | |
| 10 | | | | | | |
| 11 | | | | | | |
| 12 | | | | | | |
| 13 | | | | | | |
| 14 | | | | | | |
| 15 | | | | | | |
| 16 | | | | | | |
| 17 | | | | | | |
| 18 | | | | | | |
| 19 | | | | | | |
| 20 | | | | | | |
| 21 | | | | | | |
| 22 | | | | | | |
| 23 | | | | | | |
| 24 | | | | | | |

***Comments:***

# *Injection methods*

| injection method number | type flushed partial pick-up | Anal. time | Flush vol. | Inj. per vial | Injection volumes: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | | |
| 11 | | | | | | | | | | | | | |
| 12 | | | | | | | | | | | | | |
| 13 | | | | | | | | | | | | | |
| 14 | | | | | | | | | | | | | |
| 15 | | | | | | | | | | | | | |
| 16 | | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | | |
| 18 | | | | | | | | | | | | | |
| 19 | | | | | | | | | | | | | |
| 20 | | | | | | | | | | | | | |
| 21 | | | | | | | | | | | | | |
| 22 | | | | | | | | | | | | | |
| 23 | | | | | | | | | | | | | |
| 24 | | | | | | | | | | | | | |

***Comments:***

# *Wash methods*

| Wash method number | Wash between | Wash volume | Comments: |
|---|---|---|---|
| 1 | ☐injections<br>☐vials<br>☐series | | |
| 2 | ☐injections<br>☐vials<br>☐series | | |
| 3 | ☐injections<br>☐vials<br>☐series | | |
| 4 | ☐injections<br>☐vials<br>☐series | | |
| 5 | ☐injections<br>☐vials<br>☐series | | |

***Comments:***

# *Timebase methods*

Method number

| | Action | Time | | Action | Time |
|---|---|---|---|---|---|
| AUX 1 | 1 AUX-1 ON AT TIME: | | ISS-A | 1 ISS-A POSITION 6-1 AT TIME: | |
| | 1 AUX-1 OFF AT TIME: | | | 1 ISS-A POSITION 1-2 AT TIME: | |
| | 2 AUX-1 ON AT TIME: | | | 2 ISS-A POSITION 6-1 AT TIME: | |
| | 2 AUX-1 OFF AT TIME: | | | 2 ISS-A POSITION 1-2 AT TIME: | |
| | 3 AUX-1 ON AT TIME: | | | 3 ISS-A POSITION 6-1 AT TIME: | |
| | 3 AUX-1 OFF AT TIME: | | | 3 ISS-A POSITION 1-2 AT TIME: | |
| | 4 AUX-1 ON AT TIME: | | | 4 ISS-A POSITION 6-1 AT TIME: | |
| | 4 AUX-1 OFF AT TIME: | | | 4 ISS-A POSITION 1-2 AT TIME: | |
| AUX 2 | 1 AUX-2 ON AT TIME: | | ISS-B | 1 ISS-B POSITION 6-1 AT TIME: | |
| | 1 AUX-2 OFF AT TIME: | | | 1 ISS-B POSITION 1-2 AT TIME: | |
| | 2 AUX-2 ON AT TIME: | | | 2 ISS-B POSITION 6-1 AT TIME: | |
| | 2 AUX-2 OFF AT TIME: | | | 2 ISS-B POSITION 1-2 AT TIME: | |
| | 3 AUX-2 ON AT TIME: | | | 3 ISS-B POSITION 6-1 AT TIME: | |
| | 3 AUX-2 OFF AT TIME: | | | 3 ISS-B POSITION 1-2 AT TIME: | |
| | 4 AUX-2 ON AT TIME: | | | 4 ISS-B POSITION 6-1 AT TIME: | |
| | 4 AUX-2 OFF AT TIME: | | | 4 ISS-B POSITION 1-2 AT TIME: | |
| AUX 3 | 1 AUX-3 ON AT TIME: | | SSV | 1 SSV PORT: AT TIME: | |
| | 1 AUX-3 OFF AT TIME: | | | 2 SSV PORT: AT TIME: | |
| | 2 AUX-3 ON AT TIME: | | | 3 SSV PORT: AT TIME: | |
| | 2 AUX-3 OFF AT TIME: | | | 4 SSV PORT: AT TIME: | |
| | 3 AUX 3 ON AT TIME: | | | 5 SSV PORT: AT TIME: | |
| | 3 AUX 3 OFF AT TIME: | | | 6 SSV PORT: AT TIME: | |
| | 4 AUX 3 ON AT TIME: | | | 7 SSV PORT: AT TIME: | |
| | 4 AUX 3 OFF AT TIME: | | | 8 SSV PORT: AT TIME: | |
| AUX 4 | 1 AUX 4 ON AT TIME: | | CODE | 1 CODE-OUT: AT TIME: | |
| | 1 AUX 4 OFF AT TIME: | | | 2 CODE-OUT: AT TIME: | |
| | 2 AUX 4 ON AT TIME: | | | 3 CODE-OUT: AT TIME: | |
| | 3 AUX 4 OFF AT TIME: | | | 4 CODE-OUT: AT TIME: | |
| | 3 AUX 4 ON AT TIME: | | | 5 CODE-OUT: AT TIME: | |
| | 3 AUX 4 OFF AT TIME: | | | 6 CODE-OUT: AT TIME: | |
| | 4 AUX 4 ON AT TIME: | | | 7 CODE-OUT: AT TIME: | |
| | 4 AUX-4 OFF AT TIME: | | | 8 CODE-OUT: AT TIME: | |
| | | | END | END OF TIMED EVENTS AT: | |

# *Mix method*

| Method number: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Line | Action | Value | Position | Speed | Height | Line | Action | Value | Position | Speed | Height |
| 1 | | | | | | 41 | | | | | |
| 2 | | | | | | 42 | | | | | |
| 3 | | | | | | 43 | | | | | |
| 4 | | | | | | 44 | | | | | |
| 5 | | | | | | 45 | | | | | |
| 6 | | | | | | 46 | | | | | |
| 7 | | | | | | 47 | | | | | |
| 8 | | | | | | 48 | | | | | |
| 9 | | | | | | 49 | | | | | |
| 10 | | | | | | 50 | | | | | |
| 11 | | | | | | 51 | | | | | |
| 12 | | | | | | 52 | | | | | |
| 13 | | | | | | 53 | | | | | |
| 14 | | | | | | 54 | | | | | |
| 15 | | | | | | 55 | | | | | |
| 16 | | | | | | 56 | | | | | |
| 17 | | | | | | 57 | | | | | |
| 18 | | | | | | 58 | | | | | |
| 19 | | | | | | 59 | | | | | |
| 20 | | | | | | 60 | | | | | |
| 21 | | | | | | 61 | | | | | |
| 22 | | | | | | 62 | | | | | |
| 23 | | | | | | 63 | | | | | |
| 24 | | | | | | 64 | | | | | |
| 25 | | | | | | 65 | | | | | |
| 26 | | | | | | 66 | | | | | |
| 27 | | | | | | 67 | | | | | |
| 28 | | | | | | 68 | | | | | |
| 29 | | | | | | 69 | | | | | |
| 30 | | | | | | 70 | | | | | |
| 31 | | | | | | 71 | | | | | |
| 32 | | | | | | 72 | | | | | |
| 33 | | | | | | 73 | | | | | |
| 34 | | | | | | 74 | | | | | |
| 35 | | | | | | 75 | | | | | |
| 36 | | | | | | 76 | | | | | |
| 37 | | | | | | 77 | | | | | |
| 38 | | | | | | 78 | | | | | |
| 39 | | | | | | 79 | | | | | |
| 40 | | | | | | 80 | | | | | |

***Comments:***

# *User program*

| Method number: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Line | Action | Value | Position | Speed | Height | Line | Action | Value | Position | Speed | Height |
| 1 | | | | | | 41 | | | | | |
| 2 | | | | | | 42 | | | | | |
| 3 | | | | | | 43 | | | | | |
| 4 | | | | | | 44 | | | | | |
| 5 | | | | | | 45 | | | | | |
| 6 | | | | | | 46 | | | | | |
| 7 | | | | | | 47 | | | | | |
| 8 | | | | | | 48 | | | | | |
| 9 | | | | | | 49 | | | | | |
| 10 | | | | | | 50 | | | | | |
| 11 | | | | | | 51 | | | | | |
| 12 | | | | | | 52 | | | | | |
| 13 | | | | | | 53 | | | | | |
| 14 | | | | | | 54 | | | | | |
| 15 | | | | | | 55 | | | | | |
| 16 | | | | | | 56 | | | | | |
| 17 | | | | | | 57 | | | | | |
| 18 | | | | | | 58 | | | | | |
| 19 | | | | | | 59 | | | | | |
| 20 | | | | | | 60 | | | | | |
| 21 | | | | | | 61 | | | | | |
| 22 | | | | | | 62 | | | | | |
| 23 | | | | | | 63 | | | | | |
| 24 | | | | | | 64 | | | | | |
| 25 | | | | | | 65 | | | | | |
| 26 | | | | | | 66 | | | | | |
| 27 | | | | | | 67 | | | | | |
| 28 | | | | | | 68 | | | | | |
| 29 | | | | | | 69 | | | | | |
| 30 | | | | | | 70 | | | | | |
| 31 | | | | | | 71 | | | | | |
| 32 | | | | | | 72 | | | | | |
| 33 | | | | | | 73 | | | | | |
| 34 | | | | | | 74 | | | | | |
| 35 | | | | | | 75 | | | | | |
| 36 | | | | | | 76 | | | | | |
| 37 | | | | | | 77 | | | | | |
| 38 | | | | | | 78 | | | | | |
| 39 | | | | | | 79 | | | | | |
| 40 | | | | | | 80 | | | | | |

***Comments:***

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<LOW> 41
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